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When Does Human Life Begin? The Scientific Evidence and Terminology Revisited

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WHEN DOES HUMAN LIFE BEGIN? THE SCIENTIFIC EVIDENCE AND TERMINOLOGY REVISITED

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The question of when human life begins continues to be a source of ethical and political controversy. In this debate, the language used by many medical textbooks fosters significant misinterpretation of the scientific facts. In particular, terminology that refers to the product of sperm-egg fusion as a "penetrated oocyte" and claims that the zygote does not form until syngamy (approximately twenty-four hours after sperm-egg fusion) have resulted in the erroneous belief that a human embryo does not exist during the period prior to this point (i.e. the "pre-zygote error"). Yet an objective view of the modern scientific evidence supports only a single definition of the term "zygote": a one-cell human *organism* (i.e. a human being) that forms *immediately* upon sperm-egg fusion (not after twenty-four hours of development has elapsed). Therefore, the life of a new human being commences at a scientifically well-defined event; the fusion of the plasma membranes of sperm and egg. This conclusion is not a matter of religious belief or societal convention; it is a matter of objective, scientific observation. In light of the evidence, alternative views of when human life begins and when a developing human is the subject of rights (at viability or when the fetus is capable of conscious awareness) are both scientifically unsound and have significant negative implications for the ethical treatment of all human persons.

INTRODUCTION

A wide range of issues that are important for both science and society center on the biological and moral status of human prenatal life, including

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abortion, assisted reproduction technologies and human embryonic stem cell research. The debate over these issues often reflects deeply divided opinions. In the spring of 2013, scientists reported the successful generation of cloned human embryos that survived to the blastocyst stage before being destroyed to obtain embryonic stem cells.[1] The result was met by significant ethical objections from some commentators[2] and unqualified praise from others,[3] reflecting the widely differing views of both when human life begins and the value of human life at early embryonic stages. Similarly, a proposed amendment to the Mississippi Constitution in 2011 that would grant the rights of personhood to human embryos from the one-cell stage onward, raised considerable alarm in the media[4] and was ultimately defeated after achieving the support of a substantial number of the state's voters (42%). Similar personhood efforts in Nevada, Oklahoma, Virginia and Florida also obtained significant public support while facing strong opposition from scientists[5] and others,[6] indicating that the diversity of opinions on when human life begins noted five years ago[7] have not been resolved.

Yet even at much later stages of human prenatal development, there is no consensus on when a human embryo or fetus is a human person with basic human rights. The ambivalent public opinions surrounding the conviction of Dr. Kermit Gosnell for murder of three infants, who were killed after delivery in late-term abortions,[8] illustrates the lack of consensus in our society regarding the moral and ethical status of human prenatal life. Different religions, philosophies and cultures have come to very different

1. Masahito Tachibana et al., *Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer*, 153 CELL 1228, 1228–38 (2013).

2. *Cardinal O'Malley: Human Cloning Inconsistent with Human Dignity, Treats People as Products*, U.S. CONF. OF CATH. BISHOPS (May 13, 2013), http://www.usccb.org/news/2013/ 13–094.cfm.

3. David Cyranoski, *Human Stem Cells Created by Cloning*, 497 NATURE 295, 295–96 (2013).

4. For example, in a critical essay the New York Times stated that, if passed, the amendment "would define the term "person" in the State Constitution to include fertilized human eggs and grant to fertilized eggs the legal rights and protections that apply to people." Editorial, *The 'Personhood' Initiative*, N.Y. TIMES, Oct. 28, 2011, at A30.

5. Lee Rubin Collins & Susan L. Crockin, *Fighting 'Personhood' Initiatives in the United States*, 24 REPROD. BIOMED. ONLINE 689, 689–91 (2012); Susan Young, *Mississippi to Vote on 'Personhood'*, 479 NATURE 13, 13–14 (2011).

6. *E.g.*, Kathy Hawken, *North Dakota's Fetal Personhood Amendment: Why I Voted Against It*, THEDAILYBEAST.COM (Mar. 24, 2013), http://www.thedailybeast.com/articles/2013/03 /24/north-dakota-s-fetal-personhood-amendment-why-i-voted-against-it.html.

7. Maureen L. Condic, *When Does Human Life Begin? A Scientific Perspective*, 1 WESTCHESTER INST. WHITE PAPER 1, 1–32 (2008), *reprinted in* 9 NAT'L CATH. BIOETHICS Q. 127, 127–208 (2009).

8. *See e.g.*, Trip Gabriel & Jon Hurdle, *Philadelphia Abortion Doctor Guilty of Murder in Late-Term Procedures*, N.Y. TIMES, May 14, 2013, at A12.

conclusions on the question of when human life begins and when that life has value, leading many to conclude the question cannot be objectively resolved. Yet ample scientific evidence points to a clear resolution to this question based entirely on an objective, factual analysis of human embryonic development.

HOW DOES SCIENCE DETERMINE A NEW CELL TYPE HAS BEEN FORMED?

To address the question of when life begins from a scientific perspective, we must first consider when a new cell that is distinct from sperm and egg is formed. As previously discussed,[9] scientists determine when a new cell is formed based on two simple criteria: cell composition and cell behavior. These two criteria are used universally in the field of biology to distinguish when new cell types are produced, either in the laboratory or during embryonic development. These two factors often interact, with differences in cell composition resulting in differences in cell behavior. For example, brain cells have characteristic electrical activity required for information processing and this activity is produced by specific molecules (voltage-gated membrane channels) that are present in brain cells, but not in skin cells.

Based on both composition and behavior, it is entirely clear that a new cell type forms immediately upon sperm and egg plasma membrane fusion (Figure 1), which is a rapid event that takes less than a second to complete.[10] At this point, a single cell is generated that contains all the components of both gametes and therefore has a unique molecular composition. Moreover, the cell produced by sperm-egg fusion rapidly enters into a new pattern of cell behavior that is also distinct from either gamete (initiating cell division, for example). Thus, based on the two criteria noted above, a new cell is formed at a well-defined moment: the instant of sperm-egg plasma-membrane fusion.

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9. MAUREEN L. CONDIC ET AL., IS THIS CELL A HUMAN BEING? EXPLORING THE STATUS OF EMBRYOS, STEM CELLS AND HUMAN-ANIMAL HYBRIDS 27 (Joachim Huarte & Antoine Suarez eds., 2011); Condic, *supra* note 7.

10. Due to the singularity of the sperm-egg fusion event (which occurs only one time in one place for each oocyte) and the difficulty of obtaining oocytes (only five to ten human eggs can be harvested at a time, compared to approximately 250 million sperm in human ejaculate) the mechanisms of sperm-egg plasma membrane fusion are not well studied. However, both acrosome-reacted sperm, C.N Tomes, *Molecular Mechanisms of Membrane Fusion During Acrosomal Exocytosis*, 65 SOC'Y REPROD. FERTILITY SUPP. 275, 275–91 (2007), and mature oocytes, Min Liu, *The Biology and Dynamics of Mammalian Cortical Granules*, 9 REPROD. BIOLOGY ENDOCRINOLOGY 149, 149 (2011), express well studied SNARE and SNAP proteins on their surfaces that are likely to mediate rapid membrane fusion (~0.25 seconds) once sperm-egg binding occurs.

DEVELOPMENT REFLECTS THE ACTIVITY OF AN ORGANISM AND IS NOT CONTROLLED BY THE OOCYTE

Knowing that a new cell is formed at gamete fusion does not fully answer the question of when human life begins. We must still ask what kind of a cell has been produced—a new human being or simply a new human cell?

The medical dictionary administered by the National Institutes of Health defines the product of fertilization as a *"zygote,"* or "a cell formed by the union of two gametes; *broadly* the developing individual produced from such a cell."[11] The term "zygote" derives from the Greek *zygōtós* or "yoked," a variant of *zygoûn*, or "to join together." Thus, "zygote" is another name for a one-cell embryo that is formed by the union of sperm and egg and that undergoes the process of development to generate a mature individual.

However, there is still inherent ambiguity regarding precisely *when* the zygote forms; either at sperm-egg fusion or at fusion of the male and female pronuclei, approximately twenty-four hours later. Historically, medical texts define the formation of the zygote and the beginning human life as *syngamy*; i.e. the "fusion"[12] of the two pronuclei in preparation for the first cell division of the embryo.[13] Consequently, the new cell formed by sperm-egg fusion is often characterized as nothing more than a modified gamete (i.e. a "fertilized egg" or a "penetrated oocyte"). However, based on the ample scientific evidence[14] that is reviewed and updated here, this conclusion is clearly false. A modern understanding of human embryology indicates that syngamy does not meet either of the two criteria for the formation of a new cell. Rather, the zygote is formed in the instant of sperm-egg plasma membrane fusion, with all subsequent events of the first day of life being acts *of* the zygote, not acts that *form* the zygote (Figure 1).

The view that the zygote is simply a modified human oocyte in part reflects the difference in size and complexity between the male and female gametes. Human sperm, excluding the tail, measure roughly five microns by three microns with a volume of approximately 150 cubic microns,

11. *Zygote Definition*, MERRIAM-WEBSTER.COM, http://www.merriam-webster.com/medlineplus/zygote, April 1, 2014.

12. Although syngamy is commonly described as pronuclear "fusion," this is highly inaccurate and dangerously misleading. Condic, *supra* note 9.

13. For example, "Fertilization is a complex sequence of coordinated events that begins with contact between a sperm and an oocyte. . .and ends with the intermingling of maternal and paternal chromosomes at metaphase of the first mitotic division of the zygote." KEITH L. MOORE & T.V.N. PERSAUD, THE DEVELOPING HUMAN 31 (7th ed. 2003); "At this point, [syngamy] the process of fertilization can be said to be complete and the fertilized egg is called a zygote." BRUCE M. CARLSON, HUMAN EMBRYOLOGY AND DEVELOPMENTAL BIOLOGY 36 (3rd ed. 2004).

14. Condic, *supra* note 9.

making them one of the smallest human cell types. In contrast, a human egg is roughly 100-150 microns in diameter, having a volume of over 1.5 million cubic microns (more than ten thousand fold larger than a sperm). Consequently, the oocyte contributes the vast majority of cellular material to the zygote. This discrepancy in size, combined with the observation that an unfertilized egg can undergo many of the early steps of development when electrically stimulated and the fact that egg cytoplasm is sufficient to reprogram a mature somatic nucleus in cloning experiments, has led some to erroneously conclude that all of the factors required for early development are derived from the oocyte.

Modern evidence shows this is clearly not the case, but rather that components derived from both sperm and egg interact extensively following sperm-egg fusion to promote the ongoing development of the zygote as a whole. Based on a unique molecular composition that is distinct from an oocyte, the zygote functions immediately to direct its *own* development. Importantly, the zygote does not act like a human gamete, a human cell or even a collection of human cells, but rather like an *organism* that is undergoing a self-directed process of maturation.

An organism is distinct from a cell because all parts of an organism act together in a coordinated manner to preserve the life, health and *continued development* of the organism as a whole.[15] While human cells have complex behavior that sustains cellular life, they do not show any higher level of organization beyond that of a single cell. In contrast, human organisms exhibit globally coordinated functions that promote the health and survival of the individual as a whole. The zygote clearly exhibits such coordinated, organismal behavior from the moment of sperm-egg fusion onward. As will be illustrated by the evidence below, the activities initiated at sperm-egg fusion are not merely directed towards promoting the life of the zygote as a single cell, but rather they are directed towards *development*; i.e. production of interacting groups of cells, tissues and structures in a specific spatial and temporal sequence.

UPDATING THE EVIDENCE FOR ORGANISMAL FUNCTION OF THE EARLY HUMAN EMBRYO

The following is a summary of the events of the first five days of human life that demonstrate the organismal nature of the embryo from the moment of sperm-egg fusion onward. Some of these events (those in italics

15. An organism is defined as: "An individual constituted to carry on the activities of life by means of organs separate in function but mutually dependent: a living being." *Organism Definition*, MERRIAM-WEBSTER.COM, http://www.merriam-webster.com/medlineplus/organism, April 1, 2014.

below) have been discussed in previous works,[16] and will be mentioned only briefly here, except where new information is available. This data provides ample evidence that 1) development requires the coordinated interaction of components derived from *both* the sperm and the egg (i.e. that development is not controlled by oocyte-derived factors alone) and 2) the events of the pre-implantation period only make sense as part of an ongoing, developmental process (i.e. as activities of an organism). A summary of the scientific references for the numbered points discussed below is given in Table 1.

At Sperm-Egg Fusion

1. Sperm-egg binding: The molecular mechanism of sperm-egg binding is still not well understood. However, a large body of work has revealed critical roles in fertilization for a sperm protein (IZUMO) and an egg protein (CD9), as well as many other contributing molecules.[17] A recent study examined sperm-egg fusion in real time and showed that following sperm-egg membrane fusion, the process of incorporation of the sperm nucleus into the zygote, is very rapid with the sperm genetic material being fully engulfed by five minutes post sperm-egg fusion.[18] This demonstrates that the zygote acts rapidly to incorporate the molecular components required for its continued development.

Binding of sperm and egg also results in a rapid but poorly understood alteration of the zygote known as the "membrane block" to polyspermy, which prevents fusion of additional sperm.[19] This alteration is distinct from the cortical reaction (see point 3 below) and requires sperm-egg membrane fusion (i.e. it is not seen in cases of intracytoplasmic sperm injection or parthenogenesis[20]). Although this block is believed to develop over the first thirty minutes of life, recent evidence suggests that it may have effects as

16. Condic, *supra* note 9; Maureen L. Condic, *Totipotency: What It Is and What It Isn't*, STEM CELLS AND DEVELOPMENT (forthcoming 2014).

17. Janice P. Evans, *Sperm-egg Interaction*, 74 ANN REV. PHYSIOLOGY 477, 477–502 (2012).

18. Masahito Ikawa, Naokazu Inoue, Masaru Okabe & Yuhkoh Satouh, *Visualization of the Moment of Mouse Sperm-Egg Fusion and Dynamic Localization of IZUMO1*, 125 J. CELL SCI. 4985, 4985–90 (2012).

19. *See* Janice P. Evans & Allison J. Gardner, *Mammalian Membrane Block to Polyspermy: New Insights Into How Mammalian Eggs Prevent Fertilisation by Multiple Sperm*, 18 REPROD. FERTILITY DEV. 53, 53–61 (2006).

20. Janice P. Evans, Rafael A. Fissore, Manabu Kurokawa & Genevieve B. Wortzman-Show, *Calcium and Sperm Components in the Establishment of the Membrane Block to Polyspermy: Studies of ICSI and Activation with Sperm Factor*, 13 MOLECULAR HUM. REPROD. 557, 557–65 (2007); Michiharu Horikawa et al., *Requirement of Sperm-Oocyte Plasma Membrane Fusion for Establishment of the Plasma Membrane Block to Polyspermy in Human Pronuclear Oocytes*, 52 MOLECULAR REPROD. DEV. 183, 183–88 (1999).

early as ten seconds after sperm-egg fusion.[21] The rapid initiation of cellular modifications that prevent sperm from binding to the zygote clearly opposes the function of the gametes (whose primary purpose is to bind to each other). Moreover, blocking polyspermy is not required for the health of the zygote as a single cell, yet it is critical for embryonic development.[22] Consequently, this modification cannot be understood except as the first step of a new, *developmental* trajectory that is *immediately* initiated by the zygote to promote the health and survival of the organism as a whole.

2. Sperm entry point: Several studies[23] have pointed to the sperm entry point as a significant factor in determining the first cleavage plane of the zygote, which in turn affects how the primary body axes of the embryo are established. This conclusion has been contested by some,[24] but, if correct, it indicates that the very earliest interaction of the sperm and egg influences an event that does not occur for another twenty-four to thirty hours and supports the conclusion that sperm-egg fusion initiates a *developmental* sequence that ultimately produces the structural organization of the body as well as all the cells that the body contains.

In the first 1-5 minutes

3. Sperm-derived phospholipase-C-zeta (PLCζ): Sperm derived PLCζ is critical for the initiation of calcium oscillations that begin within the first minute following sperm-egg fusion. These oscillations will induce two important developmental events: 1) the cortical reaction that releases maternally-derived enzymes into the space surrounding the zygote to chemically modify both the zona pellucida and the cell surface so that additional sperm are unable to bind and 2) the completion of meiosis II in the oocyte-derived nucleus[25] (Figure 1). Both of these events illustrate the

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21. Yumiko Iba et al., *Possible Mechanism of Polyspermy Block in Human Oocytes Observed by Time-Lapse Cinematography*, 29 J. ASSIST. REPROD. GENET. 951, 951–56 (2012).

22. Polyploid human cells (i.e. cells having more than the normal two sets of genetic information) are common and this condition typically does not impair cellular function. In contrast, polyploidy is incompatible with embryonic development and almost always results in the early death of the embryo.

23. Karolina Piotrowska & Magdalena Zernicka-Goetz, *Role for Sperm in Spatial Patterning of the Early Mouse Embryo*, 40 NATURE 517, 517–21 (2002); Karolina Piotrowska et al., *Sperm Entry Position Provides a Surface Marker for the First Cleavage Plane of the Mouse Zygote*, 32 GENESIS 193, 193–98 (2002); Dionne Gray et al., *First Cleavage of the Mouse Embryo Responds to Change in Egg Shape at Fertilization*, 14 CURRENT BIOLOGY 397, 397–405 (2004).

24. T. J. Davies & R. L. Gardner, *The Plane of First Cleavage is not Related to the Distribution of Sperm Components in the Mouse*, 17 HUMAN REPROD. 2368 (2002); Nami Motosugi et al., *Polarity of the Mouse Embryo is Established at Blastocyst and is not Prepatterned*, 19 GENES & DEV. 1081 (2005); Sophie Louvet-Vallée et al., *Mitotic Spindles and Cleavage Planes are Oriented Randomly in the Two-Mouse Embryo*, 15 CURRENT BIOLOGY 464 (2005).

25. Sook-Young Yoon et. al., *Recombinant Human Phospholipase C Zeta 1 Induces Intracellular Calcium Oscillations and Oocyte Activation in Mouse and Human Oocytes*, 27

coordination of maternally and paternally derived factors, and only make sense as part of an ongoing developmental process that ensures the zygote will have an appropriate amount of DNA for continued, healthy maturation (i.e. these events are not required for the health of the zygote as a human cell).

4. Sperm-dependent activation of protein tyrosine kinases: Protein tyrosine kinases (PTKs) are a family of enzymes that have broad ranging effects on many cellular systems and play important roles in completion of meiosis II following fertilization.[26] Recent data has shown that sperm-egg fusion initiates a rapid localization of PTKs and an alteration of structural elements in the cortical region of the zygote beneath the sperm-entry point.[27] Although the precise role of this PTK localization and cortical modification has not yet been directly determined, an independent study indicates that removal of the sperm-associated cortical cytoplasm perturbs cell division and normal patterning of the embryo many hours later,[28] again demonstrating that sperm-egg fusion sets off a cascade of molecular events that are part of an ongoing developmental sequence.

5. Early redox state affects development: Within the first five minutes following sperm-egg fusion, calcium oscillations initiated by sperm-derived PLCζ alter the chemical oxidation-reduction (i.e. redox) reactions involved in energy metabolism of the zygote. Recent work in mice has shown that perturbing the normal sperm-induced redox state for only a few hours, beginning within the first five minutes of development, significantly alters prenatal growth, affecting the ultimate weight at birth (twenty days later) and beyond.[29] This indicates that events initiated by the zygote within minutes of sperm-egg fusion have profound and lasting effects on subsequent long-term growth and development.

In the first 30 minutes

6. Completion of meiosis: Meiosis II of the oocyte-derived nucleus is completed by thirty minutes, establishing the final diploid genome of the

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HUMAN REPROD. 1768, 1768–80 (2012); Michail Nomikos et al., *Starting a New Life: Sperm PLC-Zeta Mobilizes the Ca2+ Signal that Induces Egg Activation and Embryo Development: an Essential Phospholipase C with Implications for Male Infertility*, 34 BIOESSAYS 126 (2012).

26. Lynda K. McGinnis et al., *Localized Activation of Src-Family Protein Kinases in the Mouse Egg*, 306 DEV. BIOLOGY 241, 241–54 (2007).

27. Lynda K. McGinnis et al., *Protein Tyrosine Kinase Signaling in the Mouse Oocyte Cortex During Sperm-Egg Interactions and Anaphase Resumption*, 80 MOLECULAR REPROD. & DEV. 260, 260–72 (2013).

28. Karolina Piotrowska & Magdelenda Zernicka-Goetz, *Early Patterning of the Mouse Embryo - Contributions of Sperm and Egg*, 129 DEV. 5803, 5803–13 (2002).

29. Bernadette Banzezes et al., *Adult Body Weight is Programmed by a Redox-Regulated and Energy-Dependent Process During the Pronuclear Stage in Mouse*, 6 PLOS ONE (Dec. 28, 2011), http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0029388.

zygote. Although a third of the original triploid zygotic genome is eliminated in meiosis II, this information is nonetheless an important component of the zygote's biological state and makes significant contributions to subsequent development. The importance of this quickly eliminated DNA, and how it contributes to the development of the zygote, is illustrated by a class of genes known as "maternal effect" genes. As the name indicates, these are genes contributed by the mother that have important developmental effects even when they are absent from the final diploid genome of the zygote due to elimination at either the first or second round of meiosis. [30] These "absent" genes contribute RNA or protein to the zygote, which subsequently alters zygotic development. Often these genes have no effect at all on the normal function of the mother. This illustrates that all of the components contributed to the zygote by the oocyte and the sperm must be seen as components of the zygote itself that interact to generate a pattern of development that is unique to the specific human individual formed at sperm-egg fusion.

7. Histone H3.3 localization: Beginning within the first thirty minutes following sperm-egg fusion, protamine in the paternally derived nucleus is replaced with maternally supplied histone H3. Recent data has shown that a particular variant of this protein, histone H3.3, is preferentially localized to paternally derived DNA, while many of the unique paternal epigenetic modifications of this half of the zygotic genome are preserved.[31] The differential incorporation of histone H3.3 into the paternally derived nucleus anticipates the differential transcriptional activation of the two halves of the genome that will occur several hours later (see 12 below). Further, it demonstrates that the two halves of the zygotic genome are intimately communicating[32] well before syngamy (see point 15 below).

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30. Nicholas K. Priest & Michael J. Wade, *Maternal-Zygotic Epistasis and the Evolution of Genetic Diseases*, J. OF BIOMEDICINE AND BIOTECHNOLOGY (2010), http://dx.doi.org/10.1155/2010/478732; Ze-Xu Jiao & Theresa K. Woodruff, *Detection and Quantification of Maternal-Effect Gene Transcripts in Mouse Second Polar Bodies: Potential Markers of Embryo Developmental Competence*, 99 FERTILITY & STERILITY 2055, 2055–61 (2013); Lie Li, Ping Zheng & Jurrien Dean, *Maternal Control of Early Mouse Development*, 137 DEV. 859 (2010).

31. G. W. van der Heijden et al., *Asymmetry in Histone H3 Variants and Lysine Methylation Between Paternal and Maternal Chromatin of the Early Mouse Zygote*, 122 MECHANISMS OF DEV. 1008, 1008–22 (2005).

32. In all living cells, different parts of the genome communicate to generate coordinated patterns of cell behavior and/or development. And in all cases, this communication occurs exclusively through *indirect* mechanisms that do not require all of the cell's DNA to be physically present in the same location. The main mechanism of communication involves protein. Specific genes will be transcribed into RNA, which moves out of the nucleus into the cytoplasm, where it is translated into protein. This protein then returns to the nucleus, where it interacts with specific regions of the genome to regulate gene function. In the case of histone H3.3, a histone gene that is present in the maternally-derived half of the genome communicates with large regions of the paternally derived DNA through exactly this mechanism (i.e. the protein doesn't know or care that the paternally and maternally derived halves of the genome are in separate pronuclear

8. DNA remodeling: In addition to histone replacement, both the paternally derived and maternally derived pronuclei undergo extensive remodeling, beginning in the first thirty minutes following sperm-egg fusion. Specifically, the paternal DNA is rapidly and extensively demethylated, while the maternally derived DNA is largely protected from demethylation.[33] Recent work has implicated specific methylases[34] and other maternal factors[35] in this DNA remodeling, again indicating extensive interaction between maternal and paternal components of the zygotic genome from the earliest stages. Moreover, this remodeling (in particular, alterations in epigenetic histone marks)[36] plays an important role in the subsequent differences in transcription seen between the two pronuclei[37] (see 12 below).

In hours 1-10

9. DNA licensing as early as four hours: Cells use a molecular process known as "DNA licensing" to ensure that every chromosome is only copied once during each cell division.[38] In the zygote, the oocyte-derived nucleus localizes licensing complexes during meiosis II, but the paternally derived nucleus does not, allowing the two nuclei to have distinct patterns of DNA replication, despite existing in the same cell. This differential DNA licensing is required for nuclear division to proceed in a manner that is quite different from standard mitosis (and even from meiosis I), but entirely appropriate to the unique state of the zygote.[39]

10. DNA replication depends on sperm: Following completion of meiosis II, approximately eight to ten hours post sperm-egg fusion, both halves of the zygotic genome are replicated in anticipation of the first

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compartments). So long as the entire genome is present in a single cell (as it is in the zygote), the interactive communication between various elements of that genome proceeds in exactly this manner at all stages of life.

33. Toshinobu Nakamura et al., *PGC7/Stella Protects Against DNA Demethylation in Early Embryogenesis*, 9 NATURE CELL BIOLOGY 64, 64–71 (2007).

34. Tian-Peng Gu et al., *The Role of Tet3 DNA Dioxygenase in Epigenetic Reprogramming by Oocytes*, 477 NATURE 606, 606-10 (2011).

35. Yuki Hatanaka et al., *GSE is a Maternal Factor Involved in Active DNA Demethylation in Zygotes*, 8 PLOS ONE (Apr. 1, 2013), http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0060205.

36. Seungeum Yeo et al., *Methylation Changes of lysine 9 of Histone H3 During Preimplantation Mouse Development*, 20 MOLECULAR CELL 423, 423–28 (2005); Fátima Santos et al., *Dynamic Chromatin Modifications Characterise the First Cell Cycle in Mouse Embryos*, 280 DEV. BIOLOGY 225, 225–36 (2005).

37. Olga Østrup, Ingrid S. Anderson & Phillippe Collas, *Chromatin-Linked Determinants of Zygotic Genome Activation*, 70 CELLULAR AND MOLECULAR LIFE SCI. 1425, 1425–37 (2013).

38. Hideo Nishitani & Zoi Lygerou, *Control of DNA replication licensing in a cell cycle*, 7 GENES TO CELLS 523, 523–34 (2002).

39. Michael A. Ortega et al., *Unique Pattern of ORC2 and MCM7 Localization During DNA Replication Licensing in the Mouse Zygote*, 87 BIOLOGY OF REPROD. 1 (2012).

division of the zygote that will not occur for another fifteen hours. The onset of DNA replication is critical for normal development and is initiated by factors derived from sperm.[40] Damage to paternally derived DNA can significantly alter the timing of DNA replication,[41] indicating that coordination between maternally and paternally derived DNA controls the development of the zygote as a whole.

11. Minor wave of zygotic activation: Utilization of zygotic genes (i.e. zygotic gene transcription) is initiated in the first ten hours following sperm-egg fusion in mouse[42] and human[43] embryos, indicating that the zygote (not the mother) is controlling its own developmental progress.

12. Differential paternal-maternal transcription: The sperm-derived nucleus begins transcription earlier than the maternal nucleus[44] and is four to five fold more transcriptionally active[45] due to repression of maternal

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40. P. Comizzoli et al., *Onset of the First S-Phase is Determined by a Paternal Effect During the G1-Phase in Bovine Zygotes*, 62 BIOLOGY OF REPROD. 1677, 1677–84 (2002); L.N Eid, S.P Lorton & J.J Parrish, *Paternal Influence on S-Phase in the First Cell Cycle of the Bovine Embryo*, 51 BIOLOGY OF REPROD. 1232, 1232–37 (1994); J Schabronath & K. Gärtner, *Paternal Influence on Timing of Pronuclear DNA Synthesis in Naturally Ovulated and Fertilized Mouse Eggs*, 38 BIOLOGY OF REPROD. 744, 744–49 (1988).

41. Joanne E. Gawecka et al., *Mouse Zygotes Respond to Severe Sperm DNA Damage by Delaying Paternal DNA Replication and Embryonic Development*, 8 PLOS ONE (Feb. 19, 2013), http://www.plosone.org/; Yasushiro Yamauchi et al., *Paternal Pronuclear DNA Degradation is Functionally Linked to DNA Replication in Mouse Oocytes*, 77 BIOLOGY OF REPROD. 407, 407–15 (2007).

42. Luke Martin-Maffrey et al., *RGS14 is a Mitotic Spindle Protein Essential from the First Division of the Mammalian Zygote*, 7 DEV. CELL 763, 763–69 (2004); Christine Bouniol et al., *Endogenous Transcription Occurs at the 1-Cell Stage in the Mouse Embryo*, 218 EXPERIMENTAL CELL RESEARCH 57, 57–62 (1995); Fugaku Aoki et al., *Regulation of Transcriptional Activity During the First and Second Cell Cycles in the Preimplantation Mouse Embryo*, 181 DEV. BIOLOGY 296, 296–307 (1997); Fanyi Zeng & Richard M. Schultz, *RNA Transcript Profiling During Zygotic Gene Activation in the Preimplantation Mouse Embryo*, 283 Dev. Biology 40, 40–57 (2005).

43. Asangla Ao et al., *Transcription of Paternal Y-Linked Genes in the Human Zygote as Early as the Pronucleate Stage*, 2 ZYGOTE 281, 281–87 (1994); R. Daniels et al., *XIST Expression in Human Oocytes and Preimplantation Embryos*, 61 AM. J. HUMAN GENETICS 33, 33–39 (1997); Anthony T. Dobson et al., *The Unique Transcriptome through day 3 of Human Preimplantation Development*, 13 HUMAN MOLECULAR GENETICS 1461, 1461–70 (2004); R. Daniels et al., *Expression of the Myotonin Protein Kinase Gene in Preimplantation Human Embryos*, 4 HUMAN MOLECULAR GENETICS 389, 389–93 (1995); Morris Fiddler et al., *Expression of SRY Transcripts in Preimplantation Human Embryos*, 55 AM. J. OF MED. GENETICS 80, 80–84 (1995); R. Daniels et al., *Transcription of Tissue-Specific Genes in Human Preimplantation Embryos*, 12 HUMAN REPROD. 2251, 2251–56 (1997); Zhigang Xue et al., *Genetic Programs in Human and Mouse Early Embryos Revealed by Single-Cell RNA Sequencing*, 500 NATURE 593, 593–97 (2013).

44. Hong-Thuy Bui et al., *Essential Role of Paternal Chromatin in the Regulation of Transcriptional Activity During Mouse Preimplantation Development*, 141 REPROD. 67, 67–77 (2011); Bouniol et al., *supra* note 42; Ao et al., *supra* note 43.

45. Aoki et al., *supra* note 42; Maria Wieokowski et al., *Requirements for Promoter Activity in Mouse Oocytes and Embryos Distinguish Paternal Pronuclei from Maternal and Zygotic Nuclei*, 159 DEV. BIOLOGY 366, 366–78 (1993); Pierre G. Adenot et al., *Differential H4 Acetylation of Paternal and Maternal Chromatin Precedes DNA Replication and Differential Transcriptional Activity in Pronuclei of 1-cell Mouse Embryos*, 124 DEV. 4615, 4615–25 (1997).

transcription by sperm-derived factors.[46] Somewhat later in development, paternal genes are preferentially (although not exclusively) expressed in the placenta,[47] the earliest vital organ of the embryo. While it is clear that paternally expressed genes enhance the growth of both the placenta and the fetus,[48] it is not known whether early activation of paternal genes at the one-cell stage contributes to placenta formation. However, differential utilization of paternal and maternal genes again indicates that coordinated function of the genome begins immediately, well prior to syngamy.

In hours 10-25

13. Changes in zygote shape influence the first cleavage plane: Sperm entry establishes a specialized region of cortex in the zygote (see 2 above). Shortly after sperm-egg fusion, the zygote changes shape from a sphere to a flattened oval (Figure 2), with the site of sperm entry lying along the short axis. The first cleavage plane of the embryo aligns with this short axis and, in most cases, passes close to the site of sperm entry and the site of meiosis II (the "animal pole" of the embryo).[49] This demonstrates the earliest events of sperm-egg fusion and events that occur much later are part of an ongoing developmental sequence that has pronounced effects on the subsequent maturation of the embryo (see 16 below).

14. Position of pronuclei influences the first cleavage plane: The first cleavage plane is also influenced by the trajectory taken by the maternally and paternally derived pronuclei, and the plane they come to occupy at the center of the zygote (Figure 2), with cell division typically passing through this plane, so that the zygote is divided along what was the meridional axis of the embryo.[50] The path taken by the pronuclei is determined by the sperm

46. Bui et al., *supra* note 44.

47. S. J. Tunster et al., *Imprinted Genes in Mouse Placental Development and the Regulation of Fetal Energy Stores*, 145 REPROD. 117, 117–37 (2013); Xu Wang et al., *Paternally Expressed Genes Predominate in the Placenta*, 110 PROCEEDINGS OF THE NAT'L ACAD. OF SCI. 10705, 10705–10 (2013).

48. A. L. Fowden et al., *Imprinted Genes and the Epigenetic Regulation of Placental Phenotype*, 106 PROGRESS IN BIOPHYSIS AND MOLECULAR BIOLOGY 281, 281–88 (2011).

49. Karolina Piotrowska & Magdalena Zernicka-Goetz, *Role for Sperm in Spatial Patterning of the Early Mouse Embryo*, 409 NATURE 517, 517–21 (2011); Berenika Plusa & Karolina Piotrowska, *Sperm Entry Position Provides a Surface Marker for the First Cleavage Plane of the Mouse Zygote*, 32 GENESIS 193, 193–98 (2002); Dionne Gray et al., *First Cleavage of the Mouse Embryo Responds to Change in Egg Shape at Fertilization*, 14 CURRENT BIOLOGY 397, 397–405 (2004); R. L. Gardner, *Specification of Embryonic Axes Begins Before Cleavage in Normal Mouse Development*, 128 DEV. 839, 839–47 (2001); R. L. Gardner & T. J. Davies, *An Investigation of the Origin and Significance of Bilateral Symmetry of the Pronuclear Zygote in the Mouse*, 21 HUMAN REPROD. 492, 492–502 (2005).

50. Jing-gao Zheng et al., *Understanding Three-Dimensional Spatial Relationship Between the Mouse Second Polar Body and First Cleavage Plane with Full-Field Optical Coherence Tomography*, 18 J. BIOMEDICAL OPTICS 10503 (2013); Takashi Hiiragi & Davor Solter, *First Cleavage Plane of the Mouse Egg is not Predetermined but Defined by the Topology of the Two*

entry point and the microtubule aster produced by the sperm centrosome.[51] Zygote-specific mechanisms that involve molecules derived from both sperm and egg prevent the sperm aster from interfering with the completion of meiosis II[52] and coordinate the movement of the pronuclei to the center of the zygote once meiosis II is complete, with both events being critical for subsequent normal development of the embryo.

15. Syngamy is not the beginning of human life: As has been discussed in detail elsewhere,[53] there is no evidence for a new cell forming at syngamy; the material composition of the zygote does not change and there is no substantive change in cell behavior. All of the preparations for cell division (DNA replication, assembly of the mitotic spindle, chromatin condensation) are clearly underway hours earlier, indicating syngamy is merely another step in an already ongoing developmental process. Despite inaccurate assertions to the contrary,[54] syngamy does not form the mature genome of the zygote; the full genome of the zygote is present at sperm-egg fusion and the definitive, diploid genome is formed at the completion of meiosis II. Syngamy also does not produce a "fused" nucleus; due to nuclear membrane breakdown at syngamy (an event that occurs during every cell division at all stages of life), there is no nucleus at all. Finally, as illustrated above, the two halves of the zygotic genome do not need to be enclosed in a single nuclear membrane to interact; the zygotic genome has been functioning in a coordinated, "unified" manner since sperm-egg fusion. While syngamy is the last unique event of the first day of human life and provides further evidence for the ongoing organismal function of the embryo, it is not a "zygote-forming" event, but rather it is part of an ongoing developmental sequence that is produced by the zygote.

At the 2-3 cell stage (day two of development)

16. The overall pattern of cell division predicts cell fate: Between twenty-four and thirty hours post sperm-egg fusion, the zygote divides to produce the two-cell embryo (Figure 1E, 2C). As discussed above, in most cases the zygote divides in a manner that bisects the embryo along the animal-vegetal axis (i.e. from the position of the polar body to the position of the sperm entry point, passing through the plane of opposition of the two pronuclei; see Figure 2A, 2B). This plane of cleavage predicts the

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Apposing Pronuclei, 430 NATURE 360, 360–64 (2004).

51. Gianpiero D. Palermo et al., *The Human Sperm Centrosome is Responsible for Normal Syngamy and Early Embryonic Development*, 2 REV. REPROD. 19, 19–27 (1997).

52. Karen McNaly et al., *Kinesin-1 Prevents Capture of the Oocyte Meiotic Spindle by the Sperm Aster*, 22 DEV. CELL 788 (2012); Phuong Nguyen et al., *Pronuclear Migration: No Attachment? No Union, but a Futile Cycle*, 22 CURRENT BIOLOGY R409, R409–11 (2012).

53. Condic, *supra* note 7.

54. *See, e.g.*, Jan Tesarik & Ermanno Greco, *A Zygote is not an Embryo: Ethical and Legal Considerations*, 9 MOLECULAR ASSISTED REP. AND GENETICS 13, 13–16 (2004).

orientation of the embryonic-abembryonic axis of the embryo and the location of the inner cell mass at the blastocyst stage (Figure 2F), demonstrating that cell cleavage is part of an ongoing developmental sequence where early events influence subsequent events to produce the more mature structures of the embryo. Although the specific pattern of the second round of cell cleavage is variable (Figure 2C-2E), it strongly influences the structures each cell will produce,[55] indicating that the body plan emerges as part of a orderly developmental sequence, not as a random event.

17. The major wave of zygotic transcription is initiated at the two-cell stage: Utilization of zygotic genes increases significantly at the two-cell stage in mouse[56] and development beyond this stage requires zygotic transcription.[57] In humans, older studies suggested that the zygotic genome was not active until the four to eight cell stage,[58] yet more recent work indicates that zygotic transcription begins much earlier, with many genes being expressed at the one-cell stage (see point 11 above) and the major peak of gene activation occurring in two-cell embryos.[59] This demonstrates

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55. R. L. Gardner, *Specification of Embryonic Axes Begins Before Cleavage in Normal Mouse Development*, 128 DEV. 839 (2001); Toshihiko Fujimori et al., *Analysis of Cell Lineage in Two- and Four-Cell Mouse Embryos*, 130 DEV. 5113 (2003); Karolina Piotrowska et al., *Blastomeres Arising from the First Cleavage Division have Distinguishable Fates in Normal Mouse Development*, 128 DEV. 3739 (2001); Karolina Piotrowska-Nitsche et al., *Four-Cell Stage Mouse Blastomeres have Different Developmental Properties*, 132 DEV. 479 (2005); Shawn L. Chavez et al., *Dynamic Blastomere Behaviour Reflects Human Embryo Ploidy by the Four-Cell Stage*, 3 NATURE COMMC'N 1251 (2012).

56. Christine Bell et al., *Genomic RNA Profiling and the Programme Controlling Preimplantation Mammalian Development*, 14 MOLECULAR HUMAN REPROD. 691 (2008); Q. Tian Wang et al., *A Genome-Wide Study of Gene Activity Reveals Developmental Signaling Pathways in the Preimplantation Mouse Embryo*, 6 DEV. CELL 133 (2004); Shiho Kageyama et al., *Analysis of Transcription Factor Expression During Oogenesis and Preimplantation Development in Mice*, 15 ZYGOTE 117 (2007); Toshino Hamatani et al., *Dynamics of Global Gene Expression Changes During Mouse Preimplantation Development*, 6 DEV. CELL 117 (2004).

57. Lakshmi Rambhatla & Keith E. Latham, *Strain-Specific Progression of Alpha-Amanitin-Treated Mouse Embryos Beyond the Two-Cell Stage*, 41 MOLECULAR REPROD. & DEV. 16 (1995); P.R. Braude, *Time-Dependent Effects of Alpha-Amanitin on Blastocyst Formation in the Mouse*, 52 J. EMBRYOLOGY & EXPERIMENTAL MORPHOLOGY 193 (1979); G. M. Kidder & J. R. McLachlin, *Timing of Transcription and Protein Synthesis Underlying Morphogenesis in Preimplantation Mouse Embryos*, 112 DEV. BIOLOGY 265 (1985).

58. P. Braude et al., *Human Gene Expression First Occurs Between the Four- and Eight-Cell Stages of Preimplantation Development*, 332 NATURE 459 (1988); Jan Tesarik et al., *Early Morphological Signs of Embryonic Genome Expression in Human Preimplantation Development as Revealed by Quantitative Electron Microscopy*, 128 DEV. BIOLOGY 15 (1988).

59. Rita Vassena et al., *Waves of Early Transcriptional Activation and Pluripotency Program Initiation During Human Preimplantation Development*, 138 DEV. 3699 (2011); D. M. Taylor et al., *Paternal Transcripts for Glucose-6-Phosphate Dehydrogenase and Adenosine Deaminase are First Detectable in the Human Preimplantation Embryo at the Three- to Four-Cell Stage*, 48 MOLECULAR REPROD. & DEV. 442 (1997); Anthony Dobson et al., *The Unique Transcriptome Through Day 3 of Human Preimplantation Development*, 13 HUMAN MOLECULAR

that in both mice and humans, the zygote utilizes its own genome to direct development from the two-cell stage onward.

18. The first two blastomeres have different timing of cell division: Multiple studies have demonstrated that the first two-cells of the embryo have a bias towards different developmental fates.[60] One cell of the two-cell embryo typically divides earlier than its sister blastomere (Figure 2C) and this "leading" cell preferentially contributes to the inner cell mass, while the "lagging" cell contributes predominantly to the mural trophectoderm (Figure 2F).[61] This indicates that differences in biologic properties are present at the two-cell stage, and that these differences are part of a predictable developmental sequence that results in the non-random production of specific cell types.

19. The first two blastomeres may have different patterns of gene expression: Determining the earliest point at which blastomeres show consistent differences in gene expression is complicated by the variable patterns of cell division that are observed (see 16 and 18 above).[62] Some studies have detected no consistent differences in gene expression between the first two blastomeres,[63] while others have seen variable differences in expression of genes that regulate cell cycle kinetics[64] and still others have

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GENETICS 1461 (2004); Said Assou et al., *Dynamic Changes in Gene Expression During Human Early Embryo Development: From Fundamental Aspects to Clinical Applications*, 17 HUMAN REPROD. UPATE 272 (2011).

60. Gardner, *supra* note 55; Karolina Piotrowska et al., *Blastomeres Arising from the First Cleavage Division have Distinguishable Fates in Normal Mouse Development*, 128 DEV. 3739 (2001); Fujimori et al., *supra* note 55.

61. C.F. Graham & Z. A. Deussen, *Features of Cell Lineage in Preimplantation Mouse Development*, J. EMBRYOLOGY & EXPERIMENTAL MORPHOLOGY 53 (1978); Akiko Spindle, *Cell Allocation in Preimplantation Mouse Chimeras*, 219 J. EXPERIMENTAL ZOOLOGY 361 (1982); M. A. Surani & S.C. Barton, *Spatial Distribution of Blastomeres is Dependent on Cell Division Order and Interactions in Mouse Morulae*, 102 DEV. BIOLOGY 335 (1984); C. Louise Garbutt et al., *When and How Does Cell Division Order Influence Cell Allocation to the Inner Cell Mass of the Mouse Blastocyst?*, 100 DEV. 325 (1987).

62. For example, if the leading blastomere has a meridional division and the lagging blastomere an equatorial division (as depicted in Figure 2C), predictable cell types will be produced by each blastomere. This specific biological trajectory is likely to be associated with a specific pattern of gene activation in each cell at the two-cell stage. However, if both the leading and lagging blastomeres divide equatorially, a different developmental pattern is produced. (for review see: Cleavage pattern and emerging asymmetry of the mouse embryo, and this distinct developmental trajectory is likely to be associated with a *different* pattern of gene expression at the two-cell stage. *See* Magdalena Zernicka-Goetz, *Cleavage Pattern and Emerging Asymmetry of the Mouse Embryo*, 6 NAT. REV. MOLECULAR CELL BIOLOGY 919 (2005).) In both cases, unique genes may be expressed in each of the first two blastomeres, but since there is no way to prospectively determine what developmental trajectory the embryo will follow, these differences may not be detected in a pool of embryos at the two-cell stage that will ultimately show a variety of future developmental trajectories.

63. Matthew D. VerMilyea et al., *Transcriptome Asymmetry Within Mouse Zygotes but not between Early Embryonic Sister Blastomeres*, 4 EMBO J. 1841 (2011).

64. R. Michael Roberts et. al., *Transcript Profiling of Individual Twin Blastomeres Derived by Splitting Two-Cell Stage Murine Embryos*, 84 BIOLOGY OF REPROD. 487 (2011).

observed consistent differences in several genes important for cell signaling and cell division.[65] Thus, in addition to the distinct developmental fates noted above, it is possible that blastomeres have different molecular composition as early as the two-cell stage.

At the 4-cell stage (day 2-3 of development)

20. Differential gene expression at the four-cell stage: There is clear evidence from multiple laboratories that by the four-cell stage, blastomeres have unique patterns of gene expression that become more pronounced by the 8-cell stage.[66] This demonstrates that although cells of the embryo remain "plastic," or able to change their developmental path until the sixteen-cell stage, cells are already specialized on a molecular level by the four-cell stage.

21. Differential cell function at the four-cell stage: Associated with differences in molecular composition at the four-cell stage, there are also differences in cellular function. For example, each cell of a four-cell embryo shows a different level of transcription of zygotic genes (i.e. a difference in cell function), indicating that the contributions of the cells to subsequent development are quantitatively, and perhaps qualitatively, distinct.[67]

22. Differential developmental capability at the four-cell stage: Recombining specific blastomeres derived from four-cell embryos results in a range of developmental outcomes, from normal development to death,[68] indicating that the molecular and functional differences observed between cells at this stage also have significant developmental consequences (see Figure 2E). Importantly, these differences arise in an orderly, non-random fashion. A cell inheriting primarily vegetal cytoplasm at the second round of cell division (e.g. the cell labeled "V" in Figure 2D) subsequently shows low levels of histone modification (H3R26me2a and H3R17me2a) at the

65. Jian Hong Sun et al., *Differential Expression of Axin1, Cdc25c and Cdkn2d mRNA in 2-Cell Stage Mouse Blastomeres*, 20 ZYGOTE 305 (2012).

66. Michael Antczak & Jonathan Van Blerkom, *Oocyte Influences on Early Development: the Regulatory Proteins Leptin and STAT3 are Polarized in Mouse and Human Oocytes and Differentially Distributed within the Cells of the Preimplantation Stage Embryo*, 3 MOLECULAR HUMAN REPROD. 106 (1997); Chris Hansis et al., *Candidate Lineage Marker Genes in Human Preimplantation Embryos*, 8 REPROD. BIOMEDICINE ONLINE 577 (2004); Agnieszka Jedrusik et al., *Role of Cdx2 and Cell Polarity in Cell Allocation and Specification of Trophectoderm and Inner Cell Mass in the Mouse Embryo*, 22 GENES & DEV. 2692 (2008); Maria-Elena Torres-Padilla et al., *Histone Arginine Methylation Regulates Pluripotency in the Early Mouse Embryo*, 445 NATURE 214 (2007); Sun, *supra* note 65; Nicolas Plachta et al., *Oct4 Kinetics Predict Cell Lineage Patterning in the Early Mammalian Embryo*, 13 NATURE CELL BIOLOGY 117 (2011).

67. Maria-Elena Torres-Padilla et al., *Histone Arginine Methylation Regulates Pluripotency in the Early Mouse Embryo*, 445 NATURE 214 (2007).

68. Karolina Piotrowska-Nitsche et al., *Four-Cell Stage Mouse Blastomeres have Different Developmental Properties*, 132 DEV. 479 (2005).

four-cell stage, [69] with the progeny of these "V" cells showing high levels of Cdx2 expression at the eight-cell stage and contributing primarily to the TE lineage at the blastocyst stage.[70]

At the 8-16 cell stage (day 3-4 of development)

23. Compaction reflects a coordinated change in cell behavior: Beginning around the eight-cell stage, embryos undergo a process of compaction, where cells become polarized and adhere more tightly. As a consequence, cells located on the outside of the embryo will give rise to placenta, while inner cells will produce most of the structures of the postnatal body (Figure 2). Recent work has identified components of the molecular signaling pathway responsible for initiating this important event.[71] Surprisingly, many of the molecular players are present at earlier stages, but their function is regulated by cell-cell contacts, with inner cells being inhibited from assuming a polarized state. Independent studies showing that early cell cleavage patterns predict the segregation of cells into these two lineages (see 16 above; Figure 2) indicate that the cell-cell interactions and cell positioning at the eight-cell stage that are critical for embryo compaction are part of an ongoing developmental sequence.

24. Segregation of TE/ICM: Shortly after compaction, between the sixteen-cell and the 32-cell stage, outer and inner cells become committed to either the TE or the ICM lineage and are no longer "plastic" (i.e. able to re-specify their identities if challenged with a new set of developmental signals).[72] This indicates that by three days, the embryo has made distinct

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69. Torres-Padilla, *supra* note 67.

70. Agnieszka Jedrusik et al., *Role of Cdx2 and Cell Polarity in Cell Allocation and Specification of Trophectoderm and Inner Cell Mass in the Mouse Embryo*, 22 GENES & DEV. 2692 (2008).

71. Robert Odell Stephenson et al., *Disorganized Epithelial Polarity and Excess Trophectoderm Cell Fate in Preimplantation Embryos Lacking E-cadherin*, 137 DEV. 3383 (2010); Hiroshi Sasaki et al., *The Hippo Signaling Pathway Components Lats and Yap Pattern Tead4 Activity to Distinguish Mouse Trophectoderm from Inner Cell Mass*, 16 DEV. CELL 398 (2009); Noriyuki Nishioka et al., *Tead4 is Required for Specification of Trophectoderm in Pre-Implantation Mouse Embryos*, 125 MECHANISMS OF DEV. 270 (2008); Rieko Yagi et al., *Transcription Factor TEAD4 Specifies the Trophectoderm Lineage at the Beginning of Mammalian Development*, 134 DEV. 3827 (2007); Yoshikazu Hirate et al., *Polarity-Dependent Distribution of Angiomotin Localizes Hippo Signaling in Preimplantation Embryos*, 23 CURRENT BIOLOGY 1181 (2013); Hongjie Liu et al., *Atypical PKC, Regulated by Rho GTPases and Mek/Erk, Phosphorylates Ezrin During Eight-Cell Embryo Compaction*, 375 DEV. BIOLOGY 13 (2013).

72. Aneta Suwinska et al., *Blastomeres of the Mouse Embryo Lose Totipotency after the Fifth Cleavage Division: Expression of Cdx2 and Oct4 and Developmental Potential of Inner and Outer Blastomeres of 16- and 32-Cell Embryos*, 322 DEV. BIOLOGY 133 (2008); C. De Paepe et al., *Human Trophectoderm Cells are not Yet Committed*, 28 HUMAN REPROD. 740 (2013); R. D. Schramm & A. M. Paprocki, *In vitro Development and Cell Allocation Following Aggregation of Split Embryos with Tetraploid or Developmentally Asynchronous Blastomeres in Rhesus Monkeys*, 6 CLONING AND STEM CELLS 302 (2004); T. Shinozawa et al., *Development of Rat Tetraploid and*

cell types that will continue to interact to produce the increasingly complex tissues and structures of the maturing body.

In days 4-6

25. Coordination of hatching and implantation: In preparation for implantation, embryos must escape from the zona pellucida in a process known as hatching. TE cells are largely responsible for interacting with the uterine endometrium and therefore they must be exposed for implantation to occur. At the time of hatching, cells of the mural trophectoderm generate projections that interact with the zona pellucida.[73] Mural TE cells also secrete proteases that both digest the zona pellucida[74] and contribute to implantation.[75] The specialized function of mural TE and the coordinated changes in cell behavior associated with hatching/implantation again provide evidence for the organismal function of the early embryo.

26. Interaction of TE and ICM are critical for ongoing development: TE and ICM cannot be seen as two separate cell types that just so happen to coexist in the same place. Unlike cells of a tumor, these tissues are coordinately generated in a spatial arrangement that is appropriate to their ultimate functions. When ICM does not form or is defective, TE cells do not produce a placenta, but a tumor instead.[76] Similarly, when TE does not form or is defective, ICM cells either die[77] or do not implant.[78] This mutual dependence illustrates the organismal nature of the early embryo.

Chimeric Embryos Aggregated with Diploid Cells, 14 ZYGOTE 287 (2006); Andrezej K. Tarkowski et al., *Mouse Singletons and Twins Developed from Isolated Diploid Blastomeres Supported with Tetraploid Blastomeres*, 45 INT. J. DEV. BIOLOGY 591 (2001); Andrezej K. Tarkowski et al., *Identical Triplets and Twins Developed from Isolated Blastomeres of 8- and 16-cell Mouse Embryos Supported with Tetraploid Blastomeres*, 49 INT. J. DEV. BIOLOGY 825 (2005); Andrezej K. Tarkowski et al., *Individual Blastomeres of 16- and 32-cell Mouse Embryos are able to Develop into Foetuses and Mice*, 348 DEV. BIOLOGY 190 (2010).

73. P. B. Sesagiri et al., *Cellular and Molecular Regulation of Mammalian Blastocyst Hatching*, 83 J. REPROD. IMMUNOLOGY 79 (2009); Y. P. Cheon et al., *Role of Actin Filaments in the Hatching Process of Mouse Blastocyst*, 7 ZYGOTE 123 (1999); S. Niimura et al., *Time-lapse Videomicrographic Observations of Blastocyst Hatching in Cattle*, 56 J. REPROD. DEV. 649 (2010).

74. Rosario M. Perona & Paul W. Wasserman, *Mouse Blastocysts Hatch in Vitro by Using a Trypsin-Like Proteinase Associated with Cells of Mural Trophectoderm*, 114 DEV. BIOLOGY 42 (1986); G. V. Sireesha et al., *Role of Cathepsins in Blastocyst Hatching in the Golden Hamster*, 14 MOLECULAR HUMAN REPROD. 337 (2008).

75. N. Sharma et al., *Implantation Serine Proteinases Heterodimerize and are Critical in Hatching and Implantation*, 11 BMC DEV. BIOLOGY 61 (2006).

76. *See* Erik E. Hauzman & Zoltán Papp, *Conception without the Development of a Human Being* 36 J. OF PERINATAL MEDICINE 175 (2008); Elizabeth Garner et al., *Gestational Trophoblastic Disease*, 50 CLINICAL OBSTETRICS AND GYNECOLOGY 112 (2007).

77. K. Chawengsaksophak et al., *Homeosis and Intestinal Tumours in Cdx2 Mutant Mice*, 386 NATURE 84 (1997).

78. Alexander Meissner & Rudolf Jaenisch, *Generation of Nuclear Transfer-Derived Pluripotent ES Cells from Cloned Cdx2-deficient Blastocysts*, 439 NATURE 212 (2006).

Table 1: Scientific papers documenting the organismal functions of human embryos from the one-cell stage onward.

No.	Time	Developmental Event	Citations
1	At fusion	Sperm-egg binding	•Annu Rev Physiol. 2012;74:477-502. •J Cell Sci. 2012;125(Pt 21):4985-90. •Reprod Fertil Dev. 2006;18(1-2):53-61. •Mol Hum Reprod. 2007;13(8):557-65. •Mol Reprod Dev. 1999;52(2):183-8. •J Assist Reprod Genet. 2012;29(9):951-6.
2	At fusion	Sperm entry point	•Nature. 2001;409(6819):517-21. •Genesis. 2002;32(3):193-8. •Curr Biol. 2004;14(5):397-405.
3	1-3 minutes	Sperm PLCζ	•Hum Reprod. 2012;27(6):1768-80. •Bioessays. 2012;34(2):126-34.
4	1-3 minutes	PTK activation	•Dev Biol. 2007;306(1):241-54. •Mol Reprod Dev. 2013;80(4):260-72. •Development. 2002;129(24):5803-13.
5	1-3 minutes	Redox state	•PLoS One. 2011;6(12):e29388.
6	First 30 minutes	Meiosis II	•J Biomed Biotechnol. 2010;2010:478732. •Fertil Steril. 2013;99(7):2055-61. •Development.

			2010;137(6):859-70.
7	First 30 minutes	Histone H3.3	•Mech Dev. 2005;122(9):1008-22.
8	First 30 minutes	Paternal DNA remodeling	•Nat Cell Biol. 2007; 9: 64–71. •Nature. 2011;477(7366):606-10. •PLoS One. 2013;8(4):e60205. •Mol. Cell. 2005;20:423–428. •Dev. Biol. 2005; 280; 225–236. •Cell Mol Life Sci. 2013;70(8):1425-37.
9	1-10 hours	DNA licensing	•Biol Reprod. 2012;87(3):62.
10	1-10 hours	DNA replication; sperm factors	•Biol Reprod. 2000;62(6):1677-84. •Biol Reprod. 1994;51(6):1232-7. •Biol Reprod. 1988;38(4):744-9. •PLoS One. 2013;8(2):e56385. •Biol Reprod. 2007;77(3):407-15.
11	1-10 hours	Minor zygotic gene activation	•Dev Cell. 2004;7(5):763-9. •Exp Cell Res. 1995;218(1):57-62. •Dev Biol. 1997;181(2):296-307. •Dev Biol. 2005;283(1):40-57. •Zygote. 1994;2(4):281-7. •Am J Hum Genet. 1997;61(1):33-9. •Hum Mol Genet. 2004;13(14):1461-70. •Hum Mol Genet.

			1995;4(3):389-93. •Am J Med Genet. 1995;55(1):80-4. •Hum Reprod. 1997;12(10):2251-6. •Nature. 2013;500(7464):593-7.
12	1-10 hours	Differential DNA utilization	•Reproduction. 2011;141(1):67-77. •Exp Cell Res. 1995;218(1):57-62. •Zygote. 1994;2(4):281-7. •Dev Biol. 1997;181(2): 296-307. •Dev Biol. 1993;159(1): 366-78 •Development. 1009;124(22): 4615-25. •Reproduction. 2013;145(5):R117-37. •Proc Natl Acad Sci 2013; 25;110(26):10705-10. •Prog Biophys Mol Biol. 2011;106(1):281-8.
13	10-25 hours	Cell shape influences cleavage plane	•Nature. 2001;409(6819):517-21. •Genesis. 2002;32(3):193-8. •Curr Biol. 2004;14(5):397-405. •Development. 2001;128(6):839-47. •Hum Reprod. 2006 ;21(2):492-502.
14	10-25 hours	Pronuclei influence cleavage plane	•Biomed Opt. 2013;18(1):10503. •Nature. 2004 Jul;430(6997):360-4. •Rev Reprod. 1997; 2(1):19-27.

			•Dev Cell. 2012;22(4):788-98. •Curr Biol. 2012;22(10):R409-11.
15	10-25 hours	Syngamy	•See citation in: Natl Cathol Bioeth Quart. 2009; 9(1):127-208.
16	10-25 hours	Cell division	•Development. 2001;128: 839. •Development. 2003;130: 5113. •Development. 2001;128: 3739. •Development. 2005;132: 479. •Nat Commun. 2012;3:1251.
17	Day 2-3	Major zygotic gene activation	•Mol Hum Reprod. 2008;14(12):691-701. •Dev Cell. 2004;6(1):133-44. •Zygote. 2007;15(2):117-28. •Dev Cell. 2004;6(1):117-31. •Development. 2011;138(17):3699-709. •Mol Reprod Dev. 1997;48(4):442-8. •Hum Mol Genet. 2004;13(14):1461-70. •Hum Reprod Update. 2011;17(2):272-90.
18	Day 2-3	Leading and lagging blastomeres	•Development. 2001;128: 839–847. •Development. 2001;128:3739–3748. •Development. 2003:21:5113–5122. •J. Embryol. Exp. Morphol.

			1978;48:53–72. •J. Exp. Zool. 1982;219:361–367. •Dev. Biol. 1984;102:335–343. •Development. 1987;100:325–332.
19	Day 2-3	Differential gene expression; two-cell	•Biol Reprod. 2011;84(3):487-94. •Zygote. 2012;20(3):305-10.
20	Day 2-3	Differential gene expression; four-cell	•Mol human reproduction. 1997; 3:1067-86. •Reprod biomedicine online. 2004; 8:577-83. •Genes & development. 2008; 22:2692-706. •Nature. 2007; 445:214-8. •Zygote. 2012; 20:305-10. •Nature cell biology. 2011; 13:117-23.
21	Day 2-3	Differential cell function; four-cell	•Nature. 2007; 445:214-8.
22	Day 2-3	Differential developmental capabilities; four-cell	•Development. 2005; 132:479-90. •Nature 2007; 445:214-8. •Genes Dev. 2008;22:2692–2706.
23	Day 3-4	Compaction	•Development. 2010;137(20):3383-91. •Dev Cell. 2009;16(3):398-410. •Mech Dev. 2008;125(3-4):270-83. •Development. 2007;134(21):3827-36. •Curr Biol. 2013;23(13):1181-94. •Dev Biol. 2013;375(1):13-22.
24	Day 3-4	Commitment of	•Dev Biol. 2008; 322:133-

		TE/ICM	44. •Human reproduction. 2013; 28:740-9. •Cloning and stem cells. 2004; 6:302-14. •Zygote. 2006; 14:287-97. •Int J Dev Biol. 2001; 45:591-6. •Int J Dev Biol. 2005; 49:825-32. •Dev Biol. 2010; 348:190-8.
25	Day 4-6	Hatching and implantation	•J Reprod Immunol. 2009;83(1-2):79-84. •Zygote. 1999;7(2):123-9. •J Reprod Dev. 2010;56(6):649-54. •Dev Biol. 1986;114(1):42-52. •Mol Hum Reprod. 2008;14(6):337-46. •BMC Dev Biol. 2006;6:61.
26	Day 4-6	TE/ICM interactions	•J Perinat Med. 2008;36(2): 175. •Clinical Obs Gyn. 2007;50(1): 112. •Nature. 1997;386: 84. •Nature. 2006;439(7073): 212.

USE OF THE TERM "ZYGOTE" IN SCIENTIFIC AND MEDICAL LITERATURE

Based on the evidence discussed above, it is clear that the zygote, or one-cell human embryo, forms immediately upon sperm-egg fusion. The development of the zygote is not driven by the oocyte, but rather requires the coordinated interaction of elements derived from *both* sperm and egg. Moreover, the embryo does not function as a mere human cell or group of human cells, it functions as an *organism*; a complete human being at an immature stage of development.

In light of the clear evidence, why do so many medical texts incorrectly

identify syngamy as the beginning of human life? The erroneous conclusion that the zygote does not form until twenty-four hours post-sperm egg fusion in part reflects the definition of the term "zygote" promoted by the "Carnegie stages," a developmental staging system based on the Carnegie collection of human embryos. This system was originally published in a 1973 monograph by Ronan O'Rahilly.[79] It was revised and expanded by O'Rahilly and Müller in 1987[80] and again in 2010.[81] While O'Rahilly and Müller did not *originate* the view that the zygote is present only after syngamy,[82] their promotion of this definition has led to serious errors in how the embryo is viewed in medical literature and elsewhere.

Clearly, if a zygote is a one-cell human embryo and if one accepts the claim that the zygote does not form until approximately one day after sperm-egg fusion, it is simply a matter of logic to (erroneously) conclude that the cell existing *prior* to this point is *not* a human embryo. For example, Germany, a strongly prolife country that forbids the use of human embryos in medical research, nonetheless permits the use of "pre-zygotes" formed from fusion of sperm and egg prior to syngamy. The reason Germany allows this practice is, in agreement with the terminology of the Carnegie Stages, the human embryo is defined as "the human egg cell, fertilized and capable of development, from the time of fusion of the nuclei."[83]

The false conclusion that the human embryo does not form until twenty-four hours after sperm-egg fusion is only accentuated by the misleading Carnegie terms for stages *prior* to the formation of the zygote: the "penetrated oocyte" (at stage 1A, approximately the first thirty minutes of development) and the "ootid" (at stage 1B, approximately the first twenty-four hours prior to syngamy). Both of these terms erroneously suggest the embryo is nothing more than a modified female gamete for the

79. RONAN O'RAHILLY, *Part A: Embryos of the First Three Weeks (Stages 1 to 9), in* DEVELOPMENTAL STAGES IN HUMAN EMBRYOS, INCLUDING A SURVEY OF THE CARNEGIE (Carnegie Inst. of Washington, 1973).

80. RONAN O'RAHILLY & FABIOLA MÜLLER, DEVELOPMENTAL STATES IN HUMAN EMBRYOS, INCLUDING A REVISION OF STREETER'S "HORIZONS" AND A SURVEY OF THE CARNEGIE COLLECTION, (Carnegie Inst. of Washington, 1987).

81. Ronan O'Rahilly & Fabiola Müller, *Developmental Stages in Human Embryos: Revised and New Measurements*, 192 CELLS TISSUES ORGANS 73 (2010).

82. The term "zygote" has been used for over 100 years and there has been a long-standing emphasis on fusion of the pronuclei as a significant event in zygote formation. *See* Adam Sedgwick, *Variation and some Phenomena Connected with Reproduction and Sex*, 11 SCI. 881, 886 (1900) ("This fusion of the protoplasm of the two gametes gives us a uninucleated organism—for the fusion of the nuclei of the two gametes seems to be an essential part of the process—in which the potencies of the two gametes are blended. The *zygote*, as the mass formed of the fused gametes is called, is formed by the combination of two individualities, and is therefore essentially a new individuality, the characters of which will be different from the characters of both of the parents.").

83. Federal Embryo Protection Law, 1 Bundesgesetzblatt 2746–48 (Dec. 19, 1990).

first day of life.

Despite the misleading nature of the Carnegie terminology, it is widely used in medical texts[84] and is considered by many to be "definitive." For those who hold this view, the decision not to employ the Carnegie terminology in this analysis may appear to reflect confusion or outright ignorance. Yet this decision has been consciously made, both to avoid the false implications of O'Rahilly and Müller's definition of the zygote and to use the terminology that is, in fact, *standard* in the scientific literature.

PubMed, a service maintained by the National Institutes of Health, is the world's largest database of scientific publications, indexing more than twenty-two million research papers. A recent search of PubMed for the Carnegie term "penetrated oocyte"[85] returned only seventy-six publications out of the more than *nineteen million* publications added to the database since O'Rahilly published his original monograph in 1973 (Figure 3). Similarly, the term "ootid" has been used only six times. In contrast, searching for "zygote" (a term that has been in common usage since the 1900s[86]) returned 11,210 citations. Similarly, the term "pronuclear" (an alternative, modern name for Carnegie Stage 1B) has been used 1,868 times. A conservative search for the alternative modern terminology applied to the entire period of Carnegie stage 1 ("one-cell embryo" and variations[87]) returned 2,232 citations (Figure 3).

Searches of large databases can sometimes be misleading, generating "false negatives" where the term is actually used in the publication but does not appear in the searchable database fields. However, errors of this type are relatively unbiased for similar types of terminology (e.g. the term "pronuclear" and the term "ootid" are equally likely to be missing from the searchable database fields of a scientific publication if this stage of development is not a critical aspect of the work). Therefore, it is legitimate to compare two different terms for the same developmental stage to each other. Based on this comparison, scientists have clearly *not* adopted the terminology associated with the Carnegie Stages and, consequently, using it would be confusing to both scientific and non-scientific readers.

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84. A recent, non-scientific search returned over a thousand (primarily medical) texts and over forty thousand web pages containing the term "Carnegie stage." https://books.google.com/ngrams/graph?content=Carnegie+stage&year_start=1800&year_end=2000&corpus=15&smoothing=3&share=&direct_url=t1%3B%2CCarnegie%20stage%3B%2Cc0#t1%3B%2CCarnegie%20stage%3B%2Cc1.

85. All searches reported in this text were conducted on June 17, 2013 and are on file with the author.

86. Sedgwick, *supra* note 82.

87. A search of PubMed for (one-cell or "one-cell stage" or "one cell" or "one cell stage" or 1-cell or "1 cell") AND (embryo or embryos or embryonic). This search does not include the many variations on the general term "one-cell embryo" that are sometimes used in the literature (e.g. 1C, "single cell," unicellular, etc.).

Terminology matters in science. In discussing the topic of human embryology with non-scientists, clarity is often compromised by unnecessary precision and arcane terminology. Whenever possible, it is preferable to use terms that have clear common-sense meanings in addition to being accepted scientific terms.[88] Yet in contrast to mere lack of clarity that can be introduced by esoteric or obscure terminology, *dangerous* confusion is introduced by misleading or mistaken terminology. The Carnegie terminology, that has promoted the "pre-zygote error" by falsely suggesting an embryo does not exist for the first day of life, is a clear example of dangerously misleading terminology.

WHY DOES IT MATTER?

The scientific evidence clearly indicates that a one-cell human organism, the zygote, forms immediately at fusion of sperm and egg. From a scientific perspective, this single cell is inarguably a complete and living organism; *i.e.* a member of the human species at the earliest stage of natural development. Despite the clarity of the scientific evidence many people (including many scientists) find this conclusion difficult to accept; it seems *absurd* to call a single cell a "human being," and, consequently, many simply reject the evidence as irrelevant, and instead ask a different question: when does human life become *valuable?*

Many who question the value of the human embryo accept that human life begins at sperm-egg fusion and that human embryos are organisms, yet insist that these scientific conclusions are irrelevant to any of the important social issues that turn on the question of when life begins. What is relevant, proponents of this view assert, is the question of when the embryo acquires "rights" or "value" or "personhood," and these more nuanced and less scientifically quantifiable traits are believed to accrue gradually over developmental time.

There are two common forms of the argument that human rights/value/personhood are achieved at some point significantly later than sperm-egg fusion: the "structure/function" and the "social convention" forms. The logical implications of each argument have been considered in some detail elsewhere[89] and will be briefly discussed here.

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88. For example, the word "egg" (used in 108,147 scientific publications indexed in PubMed to refer to a non-avian female gamete) or the word "ovum" (used in 82,986 publications) are both easily understood by the public as well as being scientifically accurate terms.

89. MAUREEN L. CONDIC, *Pre-implantation Stages of Human Development: the Biological and Moral Status of Early Embryos, in* IS THIS CELL A HUMAN BEING?: EXPLORING THE STATUS OF EMBRYOS, STEM CELLS AND HUMAN-ANIMAL HYBRIDS 25, 25–44 (Antoine Suarez & Joachim Huarte eds., 2011).

Structure/Function Form: Human Rights Depend on Specific Structural and/or Functional Features

As development proceeds, a human embryo gradually acquires a familiar human form and characteristic human function. Rather than linking human rights/value to status as a human organism (a status that is clearly achieved at sperm-egg fusion), many attempt to use a specific biological structure or function as the basis for human rights, with "viability" or the development of brain structures capable of supporting "consciousness" (including the conscious perception of pain) being the most commonly invoked characteristics.

Yet both viability and higher neural function are fundamentally arbitrary. While the age of a fetus clearly affects the ability to survive following preterm birth,[90] survival also depends on a large number of factors that have nothing to do with the fetus itself, including the sophistication, proximity and affordability of neonatal intensive care facilities.[91] Consequently, linking human rights to "viability" provides an almost purely technological definition of who is and who is not the subject of basic human rights. Moreover, this definition fundamentally discriminates against those members of the human species who happen to be born in rural areas or in families without generous medical insurance policies.[92] While it is unfortunate that all infants do not have equal access to sophisticated medical care, this can hardly be the basis for determining who is a human person and who is not.

Although the attainment of "consciousness" is a less technological definition of when a developing human being has rights, the point at which the nervous system is considered to be sufficiently mature for this definition to pertain is also entirely arbitrary. The earliest neurons are born at approximately four weeks,[93] with neural activity beginning at

90. In most studies, survival at 20 weeks fetal age is approximately 10%, increasing to over 50% by 22 weeks. *See generally* Barbara J. Stoll et al., *Neonatal Outcomes of Extremely Preterm Infants from the NICHD Neonatal Research Network*, 126 PEDIATRICS 443 (2010).

91. *See* Brandon W. Alleman et al., *Individual and Center-Level Factors Affecting Morality Among Extremely Low Birth Weight Infants*, 132 PEDIATRICS 175 (2013) (indicating that in-hospital mortality rates for fetuses delivered at less than 23 weeks of fetal age varied from 28% to 90%, depending on the facility).

92. Gwieneverea D. Brandon et al., *Are Outcomes and Care Processes for Preterm Neonates Influenced by Health Insurance Status?*, 124 PEDIATRICS 122 (2009) ("In addition to the known impact of insurance status on well-being at birth, Medicaid managed care is independently associated with adverse neonatal outcomes in preterm infants.").

93. Irina Byston et al., *Tangential Networks of Precocious Neurons and Early Axonal Outgrowth in the Embryonic Human Forebrain*, 25 J. NEUROSCIENCE 2781 (2005); LIN Cheng et al., *ApoER2 and VLDLR in the Developing Human Telencephalon*, 15 EUR. J. PAEDIATR. NEUROL. 361 (2011); Irina Bystron et al., *The First Neurons of the Human Cerebral Cortex*, 9 NATURE NEUROSCIENCE 2781 (2005); Irina Bystron et al., *Development of the Human Cerebral Cortex: Boulder Committee revisited*, 9 NATURE NEUROSCIENCE 110 (2008).

approximately seven weeks and connections forming between the spinal cord and lower brain centers at approximately eighteen weeks.[94] Circuitry capable of supporting "higher" functions, such as consciousness, develops between twenty-two to twenty-four weeks.[95] Throughout this entire period, brain development is a continuous process,[96] with no clear landmarks defining when "consciousness" arises. Indeed, some have argued the brain function observed at birth is insufficient to consider a newborn infant a person.[97]

In addition to the continuous nature of brain development, it is difficult (if not impossible) to determine the qualitative mental experience of a fetus. In the debate over fetal pain legislation, for example, both sides essentially agree on the scientific fact that developing humans react to painful stimuli from approximately eight weeks of development, yet disagree on the issue of whether a fetus "perceives" pain in the same, self-reflective manner as an adult human would.[98] Consequently, those opposed to fetal pain legislation simply ignore the ample biological evidence that a fetus experiences pain in some capacity, and assert that until the fetus achieves an ill-defined level of neural sophistication that is sufficient for "awareness" of pain, this biological reaction to pain is irrelevant. Indeed, it has been argued that from a purely psychological perspective, a newborn baby does not have sufficient "life experience" to have a meaningful perception of pain until nine months after birth.[99]

The problems inherent in using any aspect of brain function as the definition of human "personhood" can be illustrated by asking just two disturbing questions. First, if we assign rights based on brain function, how do we view the wide range of variation in brain function that exists among postnatal humans? Are we comfortable assigning human rights proportionate to brain function, with the intelligent enjoying greater freedom and privilege than the less intelligent? Most of us find this idea intuitively repugnant, and yet it is entirely consistent with the logic of subordinating the rights of a fetus relative to the mother, based on

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94. N. Zecevic, *Synaptogenesis in Layer I of the Human Cerebral Cortex in the First Half of Gestation*, 8 CEREBRAL CORTEX 245 (1998).

95. M.G. Gatti et al., *Functional Maturation of Neocortex: a Base of Viability*, 25(1) J. Maternal-Fetal and Neonatal Med. 101 (2012); J. Corbett-Detig et al., *3D Global and Regional Patterns of Human Fetal Subplate Growth Determined in Utero*, 215 J. BRAIN STRUCTURE & FUNCTION 255 (2011).

96. *See Developmental Biology of Pain Perception: Hearing on* H.R. 1797 *Before the Subcomm. On the Constitution and Civil Justice*, 113th Cong. 1 (2013) (statement of Maureen L. Condic), *available at* http://www.gpo.gov/fdsys/pkg/CRPT-113hrpt109/pdf/CRPT-113hrpt109-pt1.pdf.

97. HELGA KUHSE & PETER SINGER, SHOULD THE BABY LIVE?: THE PROBLEM WITH HANDICAPPED INFANTS (Oxford Univ. Press, 1988).

98. H.R. 1797, 113th Cong. 1 (2013).

99. Stuart W.G. Derbyshire, *Can Fetuses Feel Pain?*, 332 BMJ 909 (2006).

differences in brain function.

Second, if we reject a "scaled" concept of rights, how would we identify a minimal level of brain function that qualifies an individual for human rights? While there are uniquely human neurological networks,[100] the neurological structures seen in a fetus or a newborn are very similar to other animal species.[101] Therefore it is impossible to justify assigning rights to a human infant based on distinctively human cognitive function without assigning identical rights to many animals. Indeed, because the human brain continues to develop for a very long time after birth and does not achieve mature human structure or function until between twenty and twenty-five years of age,[102] assigning rights to humans at *any* stage prior to adulthood cannot be justified based on an argument that human rights depend on the acquisition of *mature* human cognitive abilities.

Assuming it somehow proves possible to set a level of brain function or cortical connectivity that excludes human fetuses and non-human animals as holders of basic human rights while including human infants and children, there would still be significant problems with this definition. What is the status of those individuals who are initially above the bar, but subsequently fall below it as a consequence of injury, disease or old age? What about those who are born with a genetic or developmental defect that prevents them from *ever* achieving the minimum requirements? Are such individuals to be considered sub-human animals? Can they be consumed as food, used as sexual objects, exploited as sources of organs, bought and sold as property or destroyed as scientific research animals? And if not, why?

Clearly, adopting arbitrary criteria for who is the subject of rights undermines basic principles of human justice. Further if arbitrary criteria such as brain function and "viability" are insufficient to confer human rights, all other criteria based on structural or functional maturation are also similarly arbitrary and insufficient.

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100. Dante Mantini et al., *Evolutionarily Novel Functional Networks in the Human Brain?*, 33 J. NEUROSCIENCE 3259 (2013).

101. Jason Hill et al., *Similar Patterns of Cortical Expansion During Human Development and Evolution*, 107 PROCEEDINGS OF NAT'L ACAD. SCI. 13135 (2010); Tomok Sakai, *Developmental Patterns of Chimpanzee Cerebral Tissues Provide Important Clues for Understanding the Remarkable Enlargement of the Human Brain*, 280 PROCEEDINGS OF BIOLOGICAL SCI. 1753 (2012).

102. Nitin Gogtay et al., *Dynamic Mapping of Human Cortical Development During Childhood through Early Adulthood*, 101 PROCEEDINGS OF NAT'L ACAD. OF SCI. 8174 (2004); Elizabeth R. Sowell et al., *Mapping Cortical Change Across the Human Life Span*, 6 NATURE NEUROSCIENCE 309 (2003).

Social Convention Form: Rights are Assigned Based on Collective Social Judgment or Negotiation

The second common form of the argument that human rights are not intrinsically tied to status as a human being abandons the idea that rights are linked to any specific human feature, and instead argues that rights are assigned purely on the basis of pragmatic social concerns.[103] This view does not deny that an embryo or fetus is a human being, but rather asserts that society has the power to decide whether human beings have rights or not, including the right to continue living. And in many cases, the decisions of society are based on arbitrary or ill-defined criteria that nonetheless reflect social consensus.

For example, while there are real and significant differences between an adolescent and a child, the transition is gradual, with no universal landmarks defining "adolescence" for all individuals. Nonetheless, we are comfortable restricting the driving privileges of a child, while allowing adolescents to drive, based on an ill-defined and arbitrary (age-based) distinction between these two stages of human development. Similarly, it is argued, the rights of an embryo or fetus can also be restricted until some arbitrary point in maturation is achieved, even though the precise point may be impossible to objectively justify. In many cases, those arguing that rights are conferred by social convention will point to events such as viability or "consciousness" as the relevant criteria, while acknowledging there is no real change in the status of the fetus associated with these events.

Inherent to the "social convention" view is the idea that "inalienable" rights (such as the right to life, liberty and the pursuit of happiness) do not exist at all, but rather are only a matter of social accord or contract. Those who have sufficient power to impose their views or sufficient influence over public opinion to effectively promote them are free to define human "rights" in any manner they choose. Consequently, this view fatally undermines both the concept of human rights and the concept of personhood. If all rights are conferred merely by social convention, why should anyone have any rights at all beyond those they are able to acquire by persuasion, by purchase or by power? And if rights are exclusively acquired in this manner, it is inevitable that the clever, the rich and the strong will enjoy more rights than the inept, the poor and the weak. Moreover, if those with sufficient power or persuasive ability choose to *deny* rights to individuals of a particular race, religion, gender or economic class, on what basis could proponents of the 'social convention' view possibly object?

Arguments that human rights accrue gradually over developmental

103. *See* Jed Rubenfeld, *On the Legal Status of the Proposition that 'Life Begins at Conception'*, 43 STAN. L. REV. 599 (1991).

time, based either on structural/functional maturation or on social convention, are both scientifically and logically flawed. Moreover, they defy our basic concepts of justice. Therefore, criteria such as viability and consciousness should not be used as a basis for deciding when a human embryo or fetus is the subject of human rights.

In contrast, asserting that basic human rights, such as the right to life, are inherent to all human beings, regardless of their age or ability, is consistent with logic, with the facts, with our current standards of justice and with the vast majority of jurisprudence in this country. While science cannot dictate the moral and ethical status of human embryos, scientific evidence can rightly determine who is and who is not a human being and therefore who is the subject of human rights.[104] And, based on the evidence presented here, prenatal humans are clearly human beings who are entitled to basic human rights.

CONCLUSION

Modern scientific evidence demonstrates that the one-cell human embryo, or zygote, is formed at the instant of sperm-egg plasma membrane fusion. The zygote has unique material composition that is distinct from either gamete. It immediately initiates a series of cellular and biochemical events that ultimately generate the cells, tissues and structures of the mature body in an orderly temporal and spatial sequence. The capacity to undergo development is a defining characteristic of a human organism at the beginning of life. The scientific evidence presented here refutes the long-standing "pre-zygote error" promoted by the Carnegie stages that the zygote is not formed until syngamy, and therefore, that the cell produced by fusion of the gametes is nothing more than a "penetrated oocyte." Ethical positions that deny the personhood of human being at all stages of life are logically inconsistent and scientifically unsound, in addition to having significant, negative implications for the ethical treatment of all human persons.

104. Maureen L. Condic, *Science and the Politics of Personhood*, THE WITHERSPOON INSTITUTE (2012) *available at* http://www.thepublicdiscourse.com/2012/12/7300/.

GLOSSARY[105]

blastocyst: the modified blastula of a placental mammal; an early metazoan embryo typically having the form of a hollow fluid-filled rounded cavity bounded by a single layer of cells.

diploid: having the basic (haploid) chromosome number doubled. Diploid is the normal state for somatic (i.e. body) cells.

DNA: any of various nucleic acids that are usually the molecular basis of heredity, that are constructed of a double helix held together by hydrogen bonds between purine and pyrimidine bases, which project inward from two chains containing alternate links of deoxyribose and phosphate, and that in eukaryotes are localized chiefly in cell nuclei— also called deoxyribonucleic acid.

embryo: an animal in the early stages of growth and differentiation that is characterized by cleavage, the laying down of fundamental tissues, and the formation of primitive organs and organ systems; especially the developing human individual from the time of fertilization to the end of the eighth week after conception *(cleavage commences immediately after fertilization to produce the two-cell embryo).*

epigenetic: relating to, being, or involving a modification in gene expression that is independent of the DNA sequence of a gene.

fate: the expected result of normal development (i.e. what tissues or structures a cell will contribute to if left undisturbed in the embryo).

fertilization: the process of union of two gametes whereby the somatic chromosome number is restored and the development of a new individual is initiated.

gamete: a mature male or female germ cell *(sperm or egg)* usually possessing a haploid chromosome set and capable of initiating formation of a new diploid individual by fusion with a gamete of the opposite sex— also called sex cell.

gene: a specific sequence of nucleotides in DNA that is located usually on a chromosome and that is the functional unit of inheritance controlling the transmission and expression of one or more traits by specifying the structure of a particular polypeptide and especially a protein or controlling the function of other genetic material – also called determinant, determiner, factor.

genome: one haploid set of chromosomes with the genes they contain.

haploid: having the gametic number of chromosomes or half the number characteristic of somatic cells.

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105. All definitions are taken from the NIH-administered medical dictionary with minor modifications for clarity, as indicated by italics. *Available at* http://www.nlm.nih.gov/medlineplus/mplusdictionary.htm.

histone: any of various simple water-soluble proteins that are rich in the basic amino acids lysine and arginine and are complexed with DNA.

inner cell mass: *(ICM)* the portion of the blastocyst of a mammalian embryo that is destined to become the *structures of the postnatal body.*

implantation: in placental mammals: the process of attachment of the embryo to the maternal uterine wall—called also nidation.

membrane: a semipermeable limiting layer of cell protoplasm consisting of a fluid phospholipid bilayer with intercalated proteins—called also cell membrane, plasmalemma.

meiosis: the cellular process that results in the number of chromosomes in gamete-producing cells being reduced to one half and that involves a reduction division in which one of each pair of homologous chromosomes passes to each daughter cell and a mitotic division.

mitosis: a process that takes place in the nucleus of a dividing cell, involves typically a series of steps consisting of prophase, metaphase, anaphase, and telophase, and results in the formation of two new nuclei each having the same number of chromosomes as the parent nucleus.

morula: a globular solid mass of blastomeres formed by cleavage of a zygote that typically precedes the blastocyst.

nucleus: a cellular organelle of eukaryotes that is essential to cell functions (as reproduction and protein synthesis), is composed of nuclear sap and a nucleoprotein-rich network from which chromosomes and nucleoli arise, and is enclosed in a definite membrane.

organism: an individual constituted to carry on the activities of life by means of organs separate in function but mutually dependent: a living being.

ovum (oocyte, egg): a female gamete—especially a mature egg that has undergone reduction, is ready for fertilization, and takes the form of a relatively large inactive gamete providing a comparatively great amount of reserve material and contributing most of the cytoplasm of the zygote.

placenta: the vascular organ in mammals that unites the fetus to the maternal uterus and mediates its metabolic exchanges through a more or less intimate association of uterine mucosal with chorionic and usually allantoic tissues permitting exchange of material by diffusion between the maternal and fetal vascular systems but without direct contact between maternal and fetal blood and typically involving the interlocking of fingerlike vascular chorionic villi with corresponding modified areas of the uterine mucosa.

pronucleus: the haploid nucleus of a male or female gamete (as an egg or sperm) up to the time of fusion with that of another gamete in fertilization.

protamine: any of various strongly basic proteins of relatively low

molecular weight that are rich in arginine and are found, associated especially with DNA, in place of histone in the sperm of various animals.

reprogramming: altering the epigenetic state of a nucleus such that it enters into a new developmental state; e.g. during cloning, a body cell nucleus is reprogrammed by factors within an oocyte to enter into a state similar to that of a zygotic nucleus, and capable of supporting a normal pattern of embryonic development.

RNA: any of various nucleic acids that contain ribose and uracil as structural components and are associated with the control of cellular chemical activities—called also ribonucleic acid. Messenger RNA is an RNA produced by transcription that carries the code for a particular protein from the nuclear DNA to a ribosome in the cytoplasm and acts as a template for the formation of that protein—also called mRNA.

SCNT/Cloning:*Somatic cell nuclear transfer (SCNT):* transplanting nuclei from body *(i.e., somatic)* cells to enucleated eggs.

sperm (spermatozoa): *a sperm cell.* A motile male gamete of an animal usually with rounded or elongated head and a long posterior flagellum.

somatic: Of, relating to, or affecting the body especially as distinguished from the germ plasm or psyche.

syngamy: sexual reproduction by union of gametes. Commonly used to refer to the breakdown of nuclear membranes of the pronuclei approximately 24 hours after sperm-egg fusion.

transcription: the process of constructing a messenger RNA molecule using a DNA molecule as a template with resulting transfer of genetic information to the messenger RNA.

translation: the process of forming a protein molecule at a ribosomal site of protein synthesis from information contained in messenger RNA.

trophectoderm: *(TE)* the outer layer of the mammalian blastocyst after differentiation of the ectoderm, mesoderm, and endoderm when the outer layer is continuous with the ectoderm of the embryo. *Trophectoderm gives rise predominantly to the placenta.*

zona pellucida: the transparent more or less elastic noncellular glycoprotein outer layer or envelope of a mammalian ovum.

zygote: a cell formed by the union of two gametes; broadly the developing individual produced from such a cell.

Figures and Legends

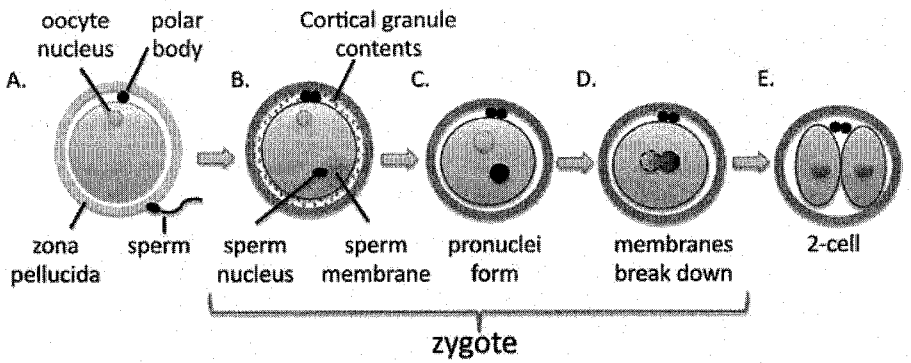

Figure 1: Coordinated events of the first day of human life. (**A**) Prior to fertilization, the oocyte nucleus has completed the first meiotic division, producing one polar body, but has not completed meiosis II. The egg is surrounded by a protective coat called the zona pellucida. (**B**) Sperm-egg fusion occurs within less than a second of sperm-egg binding, generating the *zygote*; a new cell with unique molecular composition, including sperm-derived membrane components and nucleus. Within 1-2 minutes of sperm-egg fusion, the zygote initiates changes in intracellular calcium that result in the contents of cortical granules being released into the extracellular space, modifying the zona pellucida and the surface of the zygote to prevent additional sperm from binding. Meiosis II is completed within 30 minutes. (**C**) Starting at 30 minutes, the pronuclei form by modification of the genetic material contributed by both parents. (**D**) By 24 hours, the pronuclei come together at the center of the cell, and nuclear membranes break down, an event known as syngamy. (**E**) Cell division to generate the two-cell embryo begins immediately after syngamy. **Note:** Sperm and egg are not drawn to scale. Compared to an egg of the size illustrated, a sperm would be the size of a period (.).

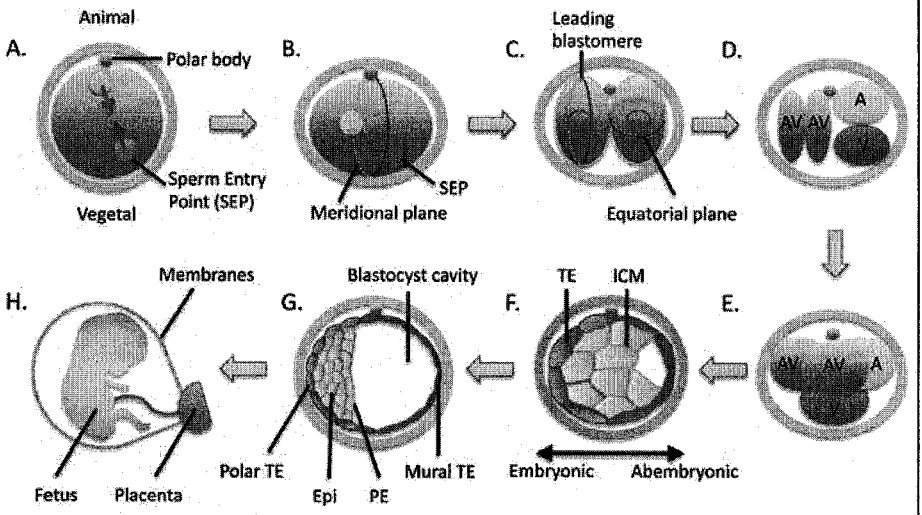

Figure 2: Preimplantation development. (**A**) The animal-vegetal (AV) axis of the zygote is defined by the position of the polar body. After sperm-egg fusion and completion of meiosis II, the maternal (pink) and paternal (blue) pronuclei move towards the center of the cell along a microtubule aster established by the sperm centrosome. (**B**) The zygote shortens along the animal-vegetal axis, and the first cell division is typically meridional, along the shorter axis in the plane defined by the position of the pronuclei and the sperm entry point. (**C**) In most cases at the two cell stage, cells have clear developmental bias, with one cell typically dividing earlier (the leading blastomere) and contributing progeny primarily to the embryonic side of the developing blastocyst (see "F" below). (**D**) One meridional and one equatorial division produces two cells that contain both animal and vegetal cytoplasmic components (AV cells), one containing primarily animal components (A cell) and one primarily vegetal components (V cell). (**E**) Once generated, cells assume a tetrahedral organization. At this stage, cells have different molecular and developmental properties. An aggregate of four AV-cells will mature to a viable mouse in 85% of cases, while four A-cells or four V-cells rarely produce a mouse [Piotrowska-Nitsche et al., *supra* note 68 (2005)]. (**F**) The AV-cells produced from the leading blastomere (see "C" above) primarily contribute to the embryonic pole of the early blastocyst, making a disproportionate contribution to the inner cell mass (ICM; green) and associated trophectoderm (TE; blue), while the "A" and "V" blastomeres will mostly contribute to abembryonic regions. (**G**) At the late blastocyst stage, the ICM has differentiated into two tissues, Epiblast (Epi; green) and primitive endoderm (PE; pink). TE associated with the ICM is termed "Polar" and that associated with the blastocyst cavity, "Mural." (**H**) The postnatal body (green) is primarily from epiblast, the placenta (blue) from TE and the membranes (pink) from both PE and TE

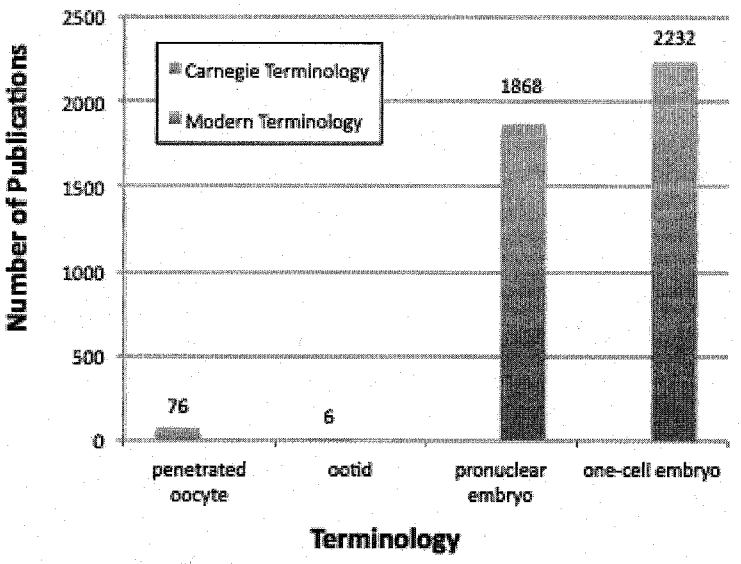

Figure 3: Usage of terminology for the first day of human development in the scientific literature. The Carnegie terminology for human embryo during the first 30 minutes is "penetrated oocyte," and during the period from 30 minutes to 24 hours, "ootid." Modern terminology for the period of 30 minutes-24 hours is "pronuclear embryo" or "one-cell embryo."