

The Significance of Fertilization*

El significado de la fecundación

C.R. AUSTIN¹

Emeritus Charles Darwin Professor of Animal Embryology
University of Cambridge, Cambridge, United Kingdom

The fertilization of a human egg, often thought of as initiating the life of a person, is in reality but the beginning of a beginning for one or more individuals. While pronuclear fusion establishes a diploid genome, this is at first a structural entity without function. No significant RNA synthesis occurs between germinal vesicle breakdown and early cleavage, and in fact embryonic genes do not begin to find expression until about the 4- to 8- cell stage. Gene expression then progressively spreads throughout the genome, during prenatal development and beyond. The progressive nature is well shown in the early mouse embryo by the widening range of energy sources utilizable, by the rising levels of glycogen storage and by the increasing uptake of nucleic acids and protein precursors. While HCG-B RNA is transcribed in human embryos about 2 days after fertilization, it is not expressed until 16-cell stage is not linked to physical or functional integration, each cell being inherently capable of giving rise to an entire person (together with a complex of placental structures); alternatively, cells from separate embryos on being brought together can jointly lead to the establishment of a chimeric individual. Multiplicity can also originate later, the primitive streak stage being the normal time for monozygotic twinning. Only when that stage has passed does true individuality exist, for (excluding anomalies) just one person can now eventuate. The gene-transfer function of fertilization can be replaced or augmented by intranuclear or intra-blastocyst gene injection, or by the use of teratocarcinoma or embryonic stem cells.

Recently in the U.S.A. a judge presiding in a Tennessee court case had to decide between the claims of an estranged couple regarding the fate of seven frozen embryos, the product of their now annulled marriage. He not only ruled that 'human life begins at conception', but also that the embryos were 'the equivalent in law children' whose best interests would be served by placing them in the care of their mother, the former wife. In this particular situation, the judge could almost be forgiven for ignoring biological facts –the climate of public opinion in his locality was strongly 'prolife' –but it is disturbing to learn that the Law should be prepared to accord such a relatively exalted status to preimplantation embryos. In many legislations, even a fullterm fetus does not have the rights of a child –not until it has been born into the world and breathed! The inconsistency is striking, and

one seeks an explanation. Perhaps this is to be found in the great advances that have been made in recent years in the maintenance of preimplantation embryos *in vitro*, because that progress has given them an important new quality, the ability to survive *independently of the mother*, for several days in culture and for an indefinite period in cryopreservation. Such a display of self-sufficiency has apparently conferred on them, at least in the minds of some people, legal rights above those commonly attributed to fetuses. Perhaps this anomalous situation will persist until we have the technology for 'ectogenesis', the total support *in vitro* of the human conceptus throughout pregnancy (a possibility discussed elsewhere: Austin, 1989). That prospect however, does not lie in the foreseeable future, so it is surely imperative under existing circumstances that the information

¹ Present Address: 47 Dixon Road, Buderim, Queensland 4556. Australia.

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before ruling on social issues involving the significance of fertilization.

Though fertilization is normally the event that initiates the developmental processes giving rise to the fetus, numerous observations make it abundantly clear that the beginning of embryonic development cannot possibly be equated with the emergence of the human *person*. The reasons relate to the functions of fertilization and the potentials for development in the cleaving embryo.

The final step in fertilization involves the union of egg and sperm chromosomes which establishes a diploid genome characteristic of the species. Initially this is purely a structural consequence, for the product shows no sign of any function as a new entity until the 4- to 8-cell stage - there is no detectable RNA synthesis by the human embryonic genome until then (Braude, Bolton & Moore, 1988). After that, evidence of function in the embryo is manifested step by step throughout development, as is well shown by the widening range of metabolites that can be utilized by the early cleaving mouse embryo (see Brinster, 1972) - from only pyruvate and oxaloacetate at the 1-cell stage to, additionally, phosphoenolpyruvate, glucose, alpha-ketoglutarate, malate and acetate at the 8-cell state. Also evident are the rising levels of glycogen storage (which increases approximately five-fold over the same period), and the mounting uptake of nucleic acids and protein precursors (most dramatic is the rise in uridine uptake - roughly 40-fold from the 2-cell state to the blastocyst). So an early but not immediate consequence of fertilization is the initiation of basic metabolic processes.

To begin with, *all* the known functions in the embryo are related to the establishment of a '*life-support system*' for sustaining the future fetus, and this is necessarily before the appearance of the fetus itself. The wall of the blastocyst (the trophoctoderm) represents the first step in placenta formation, giving rise to the chorion, which, with its proliferating villi, actively burrows into the uterine endometrium; later, groups of cells in the inner cell mass of the blastocyst will go to form the

amnion, yolk-sac, allantois and extra-embryonic coelom. About the 14th to 18th days of human pregnancy, a small group of cells lying between the amnion and yolk-sac proceed to constitute the '*embryonic disc*', which represent the primordium of the fetus itself, namely, the initial appearance of the human *person* (a process described more fully in Austin, 1990).

Against this background, the statements commonly made in public or in the media that I find most open to objection are the following:

(1) 'Human life begins at fertilization (or conception)'. It does not! For two reasons:

(a) Human eggs are, of course, alive before fertilization, but more importantly:

(b) The implication that a human *individual* is established at fertilization is quite erroneous. Individuality is emphatically *not* a feature of the embryo. The potential for *full development* that exists in *each* of the blastomeres of 2-, 4- and 8-cell embryos has been clearly demonstrated in experimental animals (e.g. Moore, Adams & Rowson, 1968) and there is no known reason why the human embryo should differ. Indeed, it has frequently been reported that clinical pregnancy can be established following the transfer from low-temperature storage of cleaving embryos in which not all (occasionally only *one* out of four) of the cells had survived. The halving or 'splitting' of morulas and blastocyst to increase the chances of pregnancy is standard practice in the highly developed cattle-embryo-transfer business. Naturally occurring human 'twinning', with the production of 'identical' twins, triplets, quadruplets, quintuplets and even sextuplets, occurs by subdivision of the embryonic disc in the 14- to 18-day-old embryo. In addition to all the above-mentioned cell separations and subdivisions, the *fusion* between two or more cleaving embryos, or between the inner cell masses of two or more blastocysts, can readily be induced (Tarkowski, 1961; Gardner, 1968), yielding single 'chimeras' capable of full development. There is clinical and genetic evidence that adult human chimeras have arisen in this way (McLaren, 1976). Taking all these events and manipulation into

account, a single fertilized human egg arguably has a developmental potential equivalent to 8x2x6, or 96, individuals, or alternatively a half or third or other fraction of *an* individual.

(2) That fertilization establishes a *unique genome*. It does not! The human genome is finite, and so no reassortment of genes can produce a unique genotype. The genomic constitution of the fertilized egg does have a high degree of *rarity*, but this property is introduced before fertilization, by the 'crossing-over' of chromosomes in the first meiotic division of the oocyte. The addition of the sperm chromosomes at fertilization does increase the degree of rarity, but the main significance of this step lies in its role in sex determination and in making possible biparental inheritance.

(3) That fertilization is a *unique mechanism* for the initiation of embryonic development. It is not! Development may also occur parthenogenetically, thus avoiding the involvement of spermatozoa (a move that could confer advantages, if it were fully successful - Austin, 1987). In the mouse, parthenogenetic embryos can progress as far as the forelimb bud stage (Kaufman, Barton & Surani, 1977), which corresponds approximately to human development of about 5 weeks. It can thus proceed to a point long after the time when the embryonic disc with its primitive groove appears. Accordingly, a person could by inference arise parthenogenetically (though significant progress in this direction has not yet been recorded).

(4) That fertilization represents the only way in which the *transfer of genes* could occur. It does not! In the laboratory, transfer can be contrived by viral infection, a mechanism that was first observed by Zinder & Lederberg (1952). Since then, viruses have been employed extensively in research on gene transfer in mammals, though more commonly investigators have used plasmids for this purpose. Particularly striking in this connection was the success

reported by Palmiter *et al.* (1982), who injected portions of rat growth hormone genes into mouse egg pronuclei and observed the subsequent birth of mice of much larger size than normal. The rat genes, in fact, became incorporated into the mouse genome, as was shown by the inheritance of the larger growth potential through two generations of progeny. This method of gene transfer has been repeated successfully in several other laboratories, and there is a distinct possibility that some such method will one day be available for correcting the genetic defects of anomalous embryos.

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