STAGE 1

Approximately 0.1-0. 15 mm in diameter
Approximately 1 postovulatory day
Characteristic feature: unicellularity

Embryonic life commences with fertilization, and hence the beginning of that process may be taken as *the point de depart* of stage 1.

Despite the small size (ca. 0.1 mm) and weight (ca. 0.004 mg) of the organism at fertilization, the embryo is "*schon ein individual-spezifischer Mensch*" (Blechschmidt, 1972). The philosophical and ethical implications have been discussed briefly by O'Rahilly and Müller (1987).

Fertilization is the procession of events that begins when a spermatozoon makes contact with an oocyte or its investments and ends with the intermingling of maternal and paternal chromosomes at metaphase of the first mitotic division of the zygote (Brackett *et al.*, 1972). Fertilization *sensu stricto* involves the union of developmentally competent gametes realized in an appropriate environment to result in the formation of a viable embryo capable of normal further development (Tesarík, 1986).

Fertilization requires probably slightly longer than 24 hours in primates (Brackett *et al.*, 1972). In the case of human oocytes fertilized *in vitro*, pronuclei were formed within 11 hours of insemination (Edwards, 1972).

Given the availability of a mature oocyte (first meiotic division completed) and capacitated spermatozoa (permitting the acrosomal reaction), the criteria for fertilization generally adopted are (1) the presence of two or more polar bodies in the perivitelline space, (2) the presence of two pronuclei within the ooplasm, and (3) the presence of remnants of the flagellum of the fertilizing spermatozoon within the ooplasm (Soupart and Strong, 1974).

Fertilization, which takes place normally in the ampulla of the uterine tube, includes (a) contact of spermatozoa with the zona pellucida of an oocyte, penetration of one or more spermatozoa through the zona pellucida and the ooplasm, swelling of the spermatozoal head and extrusion of the second polar body, (b) the formation of the male and female pronuclei, and (c)the beginning of the first mitotic division, or cleavage, of the zygote. The various details of fertilization, including such matters as capacitation, acrosomal reaction, and activation, are dealt with in special works.

When cortical granules are released, their contents appear to reinforce the structure of the zona pellucida (Sathananthan and Trounson, 1982). This is thought to be the morphological expression of the zonal reaction, and the cortical and zonal reactions may provide a block to polyspermy.

The three phases (a, b, and c) referred to above will be included here under stage 1, the characteristic feature of which is unicellularity. The sequence of events before and during the first three stages is summarized in Table l-l.

The term "ovum," which has been used for such disparate structures as an oocyte and a 3-week embryo, has no scientific usefulness and is not used here. Indeed, strictly speaking, "the existence of the ovum ... is impossible" (Franchi, 1970). The term "egg" is best reserved for a nutritive object frequently seen on the breakfast table.

At ovulation, the oocyte is a large cell surrounded by a thick covering, the zona pellucida, which is believed to be produced (at least largely) by the surrounding follicular cells. Processes of the follicular cells and microvilli of the oocyte both extend into the zona. The diameter of such a mammalian cell, including its zona, ranges from 70 to 190 µm. In the human, the ooplasm measures about 100 µm, and the thickness of the zona ranges from 16 to 18 µm (Allen *et al.*, 1930). Good photomicrographs and electron micrographs of human secondary oocytes are available (e.g., Baca and Zamboni, 1967, figs. 20 to 24; Kennedy and Donahue, 1969). The zona pellucida is covered externally by the corona radiata, which is a loose investment of granulosa cells from the ovarian follicle. On fixation

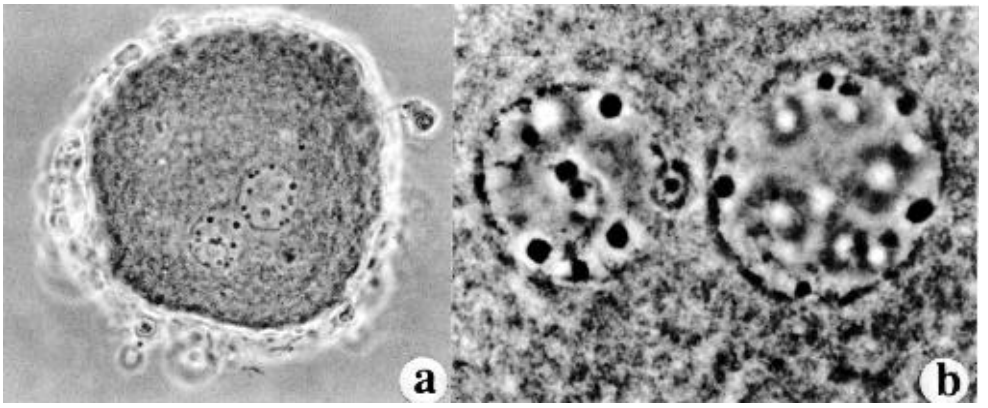

Fig. 1-1. (a) Phase contrast view of human ootid after fixation and staining. The zona pellucida had been dissolved during preparation of the specimen. (b) Phase contrast, oil immersion view of the pronuclei shown in (a). Both views, by courtesy of Dr. Z. Dickmann and Alan R. Liss, Inc. *(Anatomical Record, 152,* 293–302, 1965).

TABLE 1-1. Tabulation of the First Three Stages

Stage	Event	Products
	Meiotic division 1	Oocyte 2 and polar body 1
	Beginning of meiotic division 2 and ovulation	Ovulated oocyte
1a	Fertilization: Penetration	Penetrated oocyte
1b	Fertilization: Completion of meiotic division 2 and formation of pronuclei	Ootid and polar body 2
1c	Fertilization: Pronuclei enter cleavage division	Zygote
2	Cleavage continues	2 to about 16 cells
3	Formulation of blastocystic cavity	Blastocyst, from about 32 cells onward

and embedding, the oocyte undergoes shrinkage; this affects the cytoplasm more than the zona, so that a subzonal (or perivitelline) space becomes accentuated. The polar bodies are found within that space. It is said that the first polar body may divide before the second is released, and it has been claimed that each of the three polar bodies is capable of being fertilized. Although it is not unusual for the second polar body to display a nucleus, the chromosomes of the first polar body are isolated and naked (Zamboni, 1971).

It is "likely that no more than one day intervenes between ovulation and fertilization, This time interval may be taken then as the possible error in age of [an] embryo when it is considered the same as ovulatory age" (Rock and Hertig, 1942).

(a) Penetrated oocyte. This term may conveniently be used once a spermatozoon has penetrated the zona pellucida and, strictly, "after gamete plasma membranes have become confluent" (Zamboni *et al.,* 1966). Penetration has been inferred from the presence of spermatozoa in the zona pellucida or in the subzonal space (Edwards, Bavister, and Steptoe, 1969). Moreover, *in vitro* examples showing portions of spermatozoa within the ooplasm are illustrated by Sathananthan, Trounson, and Wood (1986), in whose work are also

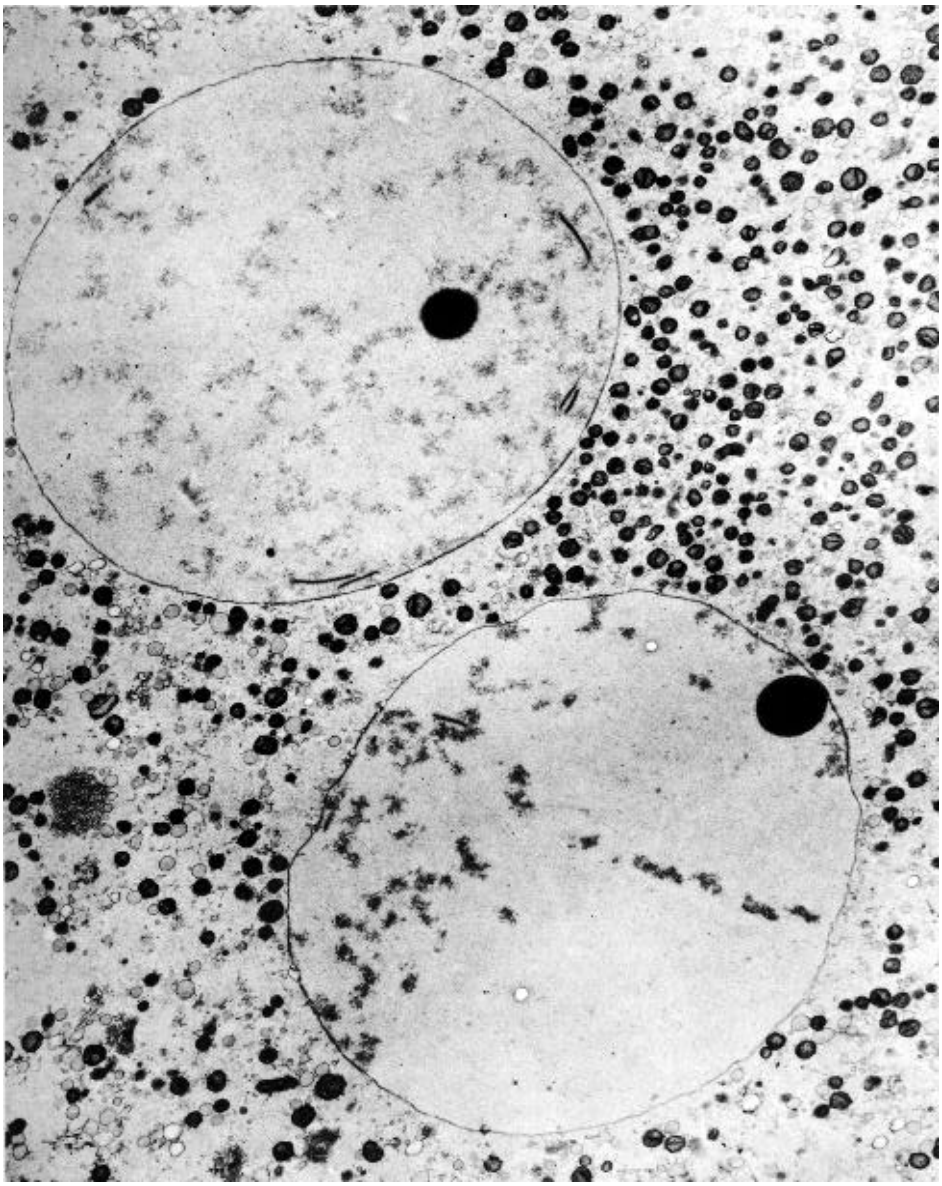

Fig. 1-2. Electron micrograph of the male and female pronuclei in a human ootid. The pronuclear material appears to be highly hydrated, although it is condensed in patches. A small black sphere, namely the nucleolus, and some annulate lamellae are visible within each pronucleus. Numerous organelles are present in the cytoplasm adjacent to the pronuclei, and portions of a Golgi complex are visible near the lower left-hand corner of the photograph. x 5,400. Reproduced through the courtesy of Dr. Luciano Zamboni, University of California, Los Angeles, and the Rockefeller University Press *(Journal of Cell Biology, 30, 579-600, 1966).*

detailed views showing the formation of the second polar body.

(*b*) *Ootid.* The cell characterized by the presence of the male and female pronuclei is termed an ootid (figs, l-l and 1-2). Several examples of human ootids have been described. They are probably about 12-24 hours in age. The diameter, including the zona pellucida, is about 175 µm (Hamilton, 1946; Dickmann *et al.*, 1965), and the diameter of the subzonal space is approximately 140 µm. The cytoplasm of the ootid has a di-

ameter of about 100 µm (Hamilton, 1946; Noyes *et al.,* 1965); each of the pronuclei measures about 30 µm (Zamboni *et al.,* 1966). The various ultrastructural features of the ootid have been described and illustrated (Zamboni *et al.,* 1966; Sathananthan, Trounson, and Wood, 1986).

Although "in most mammalian species, the male pronucleus has been reported to be larger than the female pronucleus," the converse has been found in one human specimen and, in two others, the pronuclei appeared to be of equal size (Zamboni, 1971).

(c) *Zygote*. The cell that characterizes the last phase of fertilization is elusive. The first cleavage spindle forms rapidly and has been used in identification. Such cells have probably been seen in certain mammals, e.g., the pig, cow, hamster, rat, and mouse.

Pronuclear fusion does not occur. Rather, the two pronuclear envelopes break down ("post-apposition envelope vesiculation," Szabo and O'Day, 1983), and the two groups of chromosomes move together and assume positions on the first cleavage spindle. Thus the zygote lacks a nucleus.

A human embryo "in syngamy just prior to cleavage" has been illustrated by Sathananthan and Trounson (1985, fig. 2). "The chromosomes, some associated in pairs, are located in an agranular zone in the central ooplasm."

In the human, the initial cleavage that heralds the onset of stage 2 occurs in the uterine tube "some time between twenty-four and thirty hours after [the beginning of] fertilization" (Hertig, 1968).

Specimens of Stage 1 Already Described

Embryos of stages 1-3 have been seen very frequently since the advent of *in* vitro fertilization in 1969.

Ootids have been described by the following authors:

Hamilton (1946 and 1949). Tubal. Diameter (including zona pellucida), 173 µm. Diameter of ooplasm, 100 µm. Sectioned serially at 7 µm. Two pronuclei, one larger than the other. Many spermatozoa in zona pellucida. Dickmann *et al.* (1965) have expressed some doubts about this specimen.

Khvatov (1959). Tubal. Two pronuclei, claimed to be distinguished as male and female.

Dickmann *et al.* (1965). Tubal. Diameter (including zona), 174 µm. Zona pellucida, 17.5 µm in thickness. Diameter of ooplasm, 103 µm (Noyes *et al.,* 1965). Two pronuclei, approximately equal in size (fig. l-lb). Nucleoli visible. Tail of fertilizing spermatozoon identified over one pronucleus. Well illustrated (figs. l-la and b).

Zamboni *et al.* (1966). Tubal. Ootid estimated to have a maximum diameter of about 150 µm, and 110-120 µm without the zona pellucida (Zamboni, personal communication, 1970). Fixed and sectioned for electron microscopy. Zona seen and three polar bodies identified. Two pronuclei, of about equal size (30 µm), each with a spheroidal nucleolus. Remnants of penetrating spermatozoon identified near one pronucleus. Ultrastructural findings described in detail and well illustrated (fig. l-2).

Edwards, Bavister, and Steptoe (1969). Seven ootids resulted from insemination *in* vitro of oocytes matured *in vitro*. Two had two pronuclei each, four had three each, and one had five. Photographs, but no cytological details, were provided.

Soupart and Morgenstern (1973). Two pronuclei and two polar bodies obtained in vitro.

Soupart and Strong (1974). Fourteen examples examined by electron microscopy. Two pronuclei (that near spermatozoal flagellum believed to be male) and two polar bodies.

Lopata *et al.* (1978, 1980). Several in *vitro* examples.

Sathananthan and Trounson (1982) studied the release of cortical granules at stages 1 and 2.

Pereda and Coppo (1984) found, by electron microscopy, light and dark follicular cells surrounding an ootid from the uterine tube.

Sathananthan, Trounson, and Wood (1986). Several in vitro examples are illustrated.