FERTILITY AND STERILITY
Vol. 26, No. 12, December 1975
Printed in U.S.A.

A SEVEN-CELL HUMAN EGG RECOVERED FROM THE OVIDUCT*

SERGIO AVENDAÑO, M.D., HÉCTOR D. CROXATTO, M.D., JAIME PEREDA, PH.D., AND HORACIO B. CROXATTO, M.D.†

Hospital Barros Luco-Truddeau, Universidad de Chile, and Laboratorio de Endocrinología, Universidad Católica de Chile, Santiago, Chile

The preimplantation stages of human development have been only partially characterized in the reports of 19 fertilized eggs. Eight of these eggs were recovered from the oviducts and eleven from the uterus. Of the oviductal eggs, four were in the pronuclear stage,[1-4] one was in the two-cell stage,[5] and one was in the four-cell stage.[6] The other two[7,8] had a number of cells unusually large for oviductal eggs. One of these eggs, composed of more than 200 cells, "was washed from a partially blocked human tube."[8] It is likely that, in both cases, egg development proceeded beyond the normal stage in spite of some condition preventing their passage into the uterus. Of the uterine eggs, four were considered abnormal; the others had 12,[5,9] 16,[10] 58,[5] 107,[5] and 186[10] cells, respectively.

The present paper describes a seven-cell human egg, thus representing an egg in the most advanced developmental stage recovered from the oviduct under reasonably normal conditions. A partial account of the ultrastructure of this egg has been reported.[11]

Received April 7, 1975.

*Undertaken as part of the Contraceptive Development Research Program sponsored and coordinated by the International Committee for Contraception Research of The Population Council, New York, N. Y.

†Reprint requests: Horacio B. Croxatto, M.D., Laboratorio de Endocrinología, Instituto de Ciencias Biológicas, Universidad Católica de Chile, Casilla 114-D, Santiago, Chile.

MATERIALS AND METHODS

The egg was obtained from a normal, untreated 30-year-old woman, gravida 5, para 5, who had requested surgical sterilization. She had given her consent to collaborate in a study of egg transport.

Luteinizing hormone (LH) was measured by hemagglutination inhibition assay (Luteonosticon, Oss, Holland) in the first morning urine specimen collected during each of 5 days preceding surgery. Cervical mucus was examined microscopically at frequent intervals between the last menstrual period and the day of surgery, in order to assess the changes in its physical properties and to detect the occurrence of insemination.

The operation consisted of the following steps: laparotomy, bilateral salpingectomy, ovarian biopsy, flushing of the endometrial cavity, and endometrial biopsy. The oviducts were divided into four equal segments which were flushed separately with Tyrode's solution. The flushings obtained from the oviducts and uterus were examined under low power with a dissecting microscope, in a search for the egg. The oviductal segments were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin for subsequent identification. The egg was photographed in Tyrode's solution in 10 different focal planes and then fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 2% osmium tetroxide, and embedded in Epon 812.[12] Sections

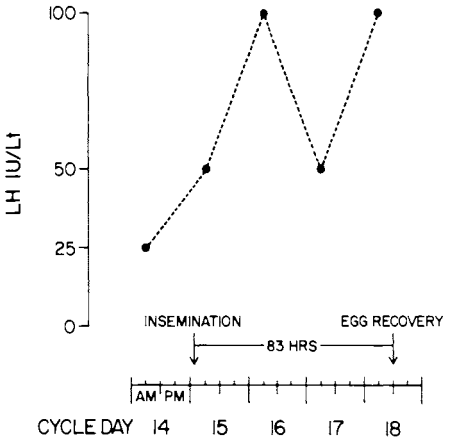

FIG. 1. Timing of events preceding the recovery of a seven-cell egg from the human oviduct.

varying from 0.5 to 1.0 μm in thickness were stained with toluidine blue.

RESULTS

The timing of the events is shown in Figure 1. The operation was performed at approximately noon on the 18th day of the cycle. The patient reported having had intercourse at 1:00 A.M. on the 15th day, which was 83 hours before the egg was recovered. The possible occurrence of previous or subsequent inseminations was ruled out by the results of microscopic examination of the cervical mucus.

The first significant elevation of the LH level in the urine was detected 77 hours prior to egg recovery and was followed by another increase of up to 100 IU/liter 24 hours later. From then on, although there was some fluctuation, the LH concentration remained above the basal level until the day of surgery.

The egg was found in the flushings which had been obtained from the proximal middle quarter of the right oviduct. The segment from which the egg was flushed was identified histologically as ampulla (Fig. 2).

The egg was completely denuded of granulosa cells, which facilitated the microscopic appraisal of the components of the fresh specimen (Fig. 3). However, the photographic documentation was limited practically to one angle, since the

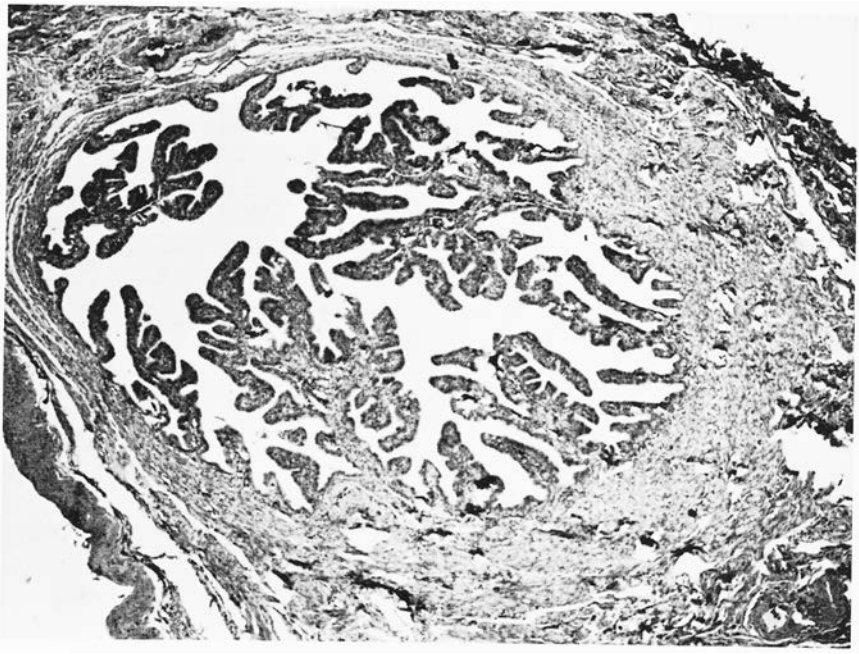

FIG. 2. Cross-section of oviductal segment which contained the egg, between 5 and 7.5 cm from the fimbriated end (×46).

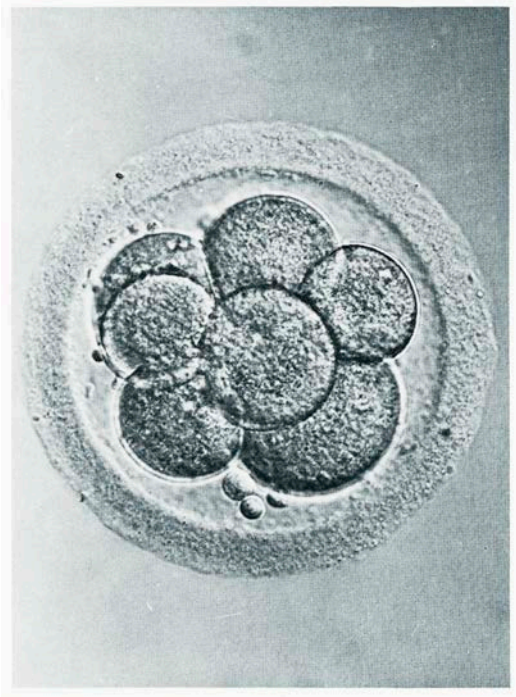

FIG. 3. Seven-cell human egg photographed before fixation. Three polar bodies are clearly seen in the lower part, next to the larger blastomere (×312).

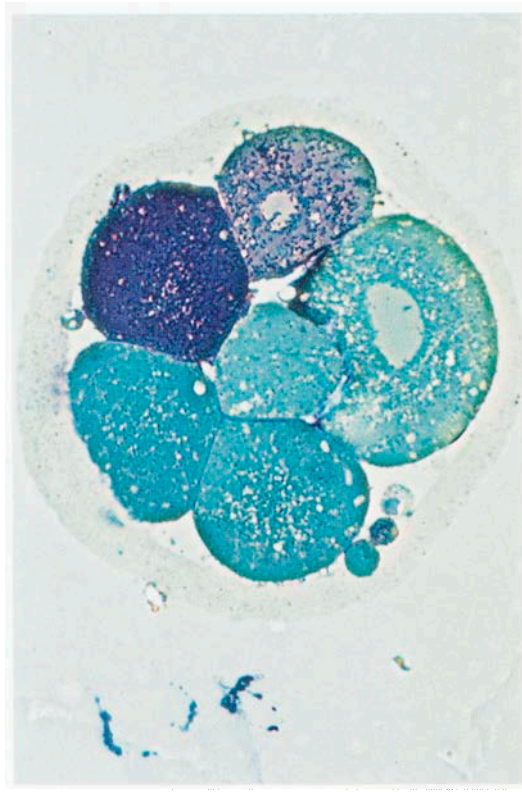

FIG. 4. Photomicrograph of a section of the egg stained with toluidine blue. The nuclei of two of the blastomeres are clearly visible. Two adjacent blastomeres and one of the polar bodies exhibit a metachromatic reaction (×390).

egg was markedly flattened, resembling a disc, and tended to return quickly to the same configuration. The dimensions were 201 × 197 μm. The third dimension could not be measured accurately, but it was estimated not to exceed 175 μm. The zona pellucida was intact, measuring 25 μm in thickness, and had a fine granular appearance. There was at least one sperm adhering to the surface. The perivitelline space was narrow and clear. Three polar bodies were readily recognized in the perivitelline space, adjacent to a large blastomere. Under bright-field, four to five rounded masses smaller than the polar bodies could be seen; with phase-contrast, much finer particulate matter was also recognized.

Seven blastomeres were counted. They were arranged in two groups occupying different focal planes. At the same time, blastomeres of four different sizes were seen. One group was formed by one large blastomere (76 μm) and three of intermediate size (65, 64, and 59 μm). The other group was in the same focal plane as the polar bodies and was formed by one blastomere of intermediate size (57 μm) occupying a central position with two of the smaller size (53 and 53 μm).

The four blastomeres which were in the same focal plane showed a flat contour along their contact surfaces. However, the two smaller blastomeres showed a concave contour in their zone of contact with the one centrally located.

Photographs of the sections stained with toluidine blue were taken at an angle slightly different from that of the photographs of the fresh specimen. Two blastomeres showed a metachromatic reaction. The nuclei of two blastomeres and of the

FIG. 5. Endometrial biopsy specimen showing abundant and irregular vacuolation of the glandular epithelium (×280).

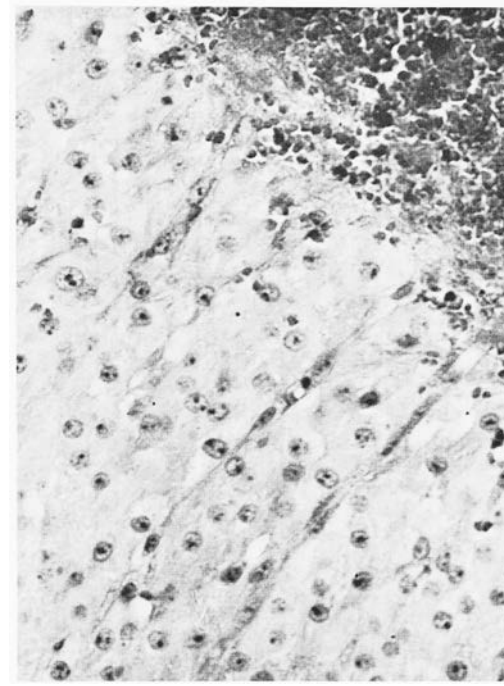

FIG. 6. Corpus luteum biopsy specimen, luteinized and well-vascularized granulosa layer. Some capillaries have reached the central cavity, which is occupied by a recent hemorrhage (*upper right*) (×352).

polar bodies were photographed in these sections (Fig. 4).

The endometrial biopsy corresponded to that of a secretory endometrium on day 17 of a 28-day cycle (Fig. 5). The biopsy specimen obtained from the right ovary contained a corpus luteum in its 4th day of development (Fig. 6).

DISCUSSION

The structure described could be a normally developing seven-cell egg ready to complete the third cleavage division. No definitely abnormal features were apparent. However, the normal human egg at this stage of development has not been previously characterized. Therefore, until additional specimens are studied, it is difficult to judge the present one. The features which led Hertig and associates[5] to classify four of their preimplantation embryos as abnormal were not found here. Although the pronounced flattening of the present egg may be an abnormal feature, this is a premature conclusion. The particulate matter may represent remnants of corona cell macrovilli, which have been observed in the perivitelline space in cultivated follicular oocytes[13] as well as in human tubal ova.[14] The egg was definitely fertilized, as indicated by the presence of three polar bodies and of nucleated blastomeres.

The age of the egg could not exceed 83 hours, according to the insemination time recorded. Furthermore, the histologic dating of the endometrium and corpus luteum limits the age to 72 hours. Finally, the LH levels in urine limit the age to less than 72 hours, a period compatible with the stage of development.

The two metachromatic blastomeres could represent true differentiation. Alternatively, they may simply be in dif-

ferent stages of an asynchronous cycle of cellular division.

The spatial arrangement of the blastomeres is such that each one seemed to be in contact with four others, with two exceptions: (1) the one centrally located, which could well have contact with the other six, unless a small cavity was already present; (2) a small blastomere, the one located next to the larger one, which was related to only three others.

The differences in size are difficult to interpret. The largest blastomere is probably the one that had not completed the third cleavage division, thus explaining the uneven number. This would indicate that there was already some degree of asynchrony at this early stage of development. One could further assume that the three which had already undergone the third cleavage division had done so in varying manners. Two of them had each given rise to a large (64 or 65 μm) and a small (53 μm) blastomere, and the third one to two almost equal blastomeres (57 to 59 μm). This uneven division would produce no ultimate loss or gain of mass in the process of cell division. Unless the differences in size are an indication of abnormal development, these findings suggest a very early gross differentiation in the human egg.

SUMMARY

A seven-cell human egg recovered from the proximal middle quarter of the oviduct is described. Whether or not it is a normal representative of this stage of human development cannot be established at the present time. The specimen was recovered 83 hours after intercourse and 77 hours after the first significant elevation of the luteinizing hormone level in the urine. According to these data and the results of the endometrial and corpus luteum biopsies, the age of the egg was estimated to be approximately 72 hours. An analysis of size and the reaction of the blastomeres to toluidine blue suggests that they already show some differentiation at this early stage of development. The addition of these findings to previous reports of eggs recovered from human oviducts and uteri gives support to the concept that human eggs are delivered to the endometrial cavity when they contain between 7 and 12 blastomeres.

Acknowledgment. The authors are indebted to Dr. Arthur T. Hertig, Shattuck Professor of Pathological Anatomy, Emeritus, Harvard University, for his expert appraisal of the biopsy material in this work. His diagnosis was "endometrium and corpus luteum consistent with the late third or early fourth day following ovulation."

REFERENCES

1. Hamilton WJ: Early stages of human development. Ann R Coll Surg Engl 4:281, 1949
2. Khvatov B: Fertilization and early development of human ova in the tubes. Anat Rec 136:222, 1960
3. Dickmann Z, Clewe RH, Bonney WA, Noyes RW: The human egg in the pronuclear stage. Anat Rec 152:293, 1965
4. Zamboni L, Mishell DR, Bell JH, Baca M: Fine structure of the human ovum in the pronuclear stage. J Cell Biol 30:579, 1966
5. Hertig AT, Rock J, Adams EC, Mulligan W: On the preimplantation stages of the human ovum: a description of four normal and four abnormal specimens ranging from the second to the fifth day of development. Contrib Embryol 35:199, 1954
6. Doyle LL, Lippes J, Winters HS, Margolis AJ: Human ova in the fallopian tube. Am J Obstet Gynecol 95:115, 1966
7. Khvatov BP: Human embryo at the stage of blastodermic vesicle. Arch Anat Histol Embryol 53:51, 1967. Cited by O'Rahilly R: In Developmental Stages in Human Embryos, Part A: Embryos of the First Three Weeks (Stages 1 to 9). Washington D C, Carnegie Institution Publication 631, 1973
8. Krafka J: A free tubal ovum in a late cleavage stage. Anat Rec 82:426, 1942
9. Croxatto HB, Fuentealba B, Diaz S, Pastene L, Tatum HJ: A simple nonsurgical technique to obtain unimplanted eggs from human uteri. Am J Obstet Gynecol 112:662, 1972

10. Croxatto HB, Díaz S, Fuentealba B, Croxatto HD, Carrillo D, Fabres C: Studies on the duration of egg transport in the human oviduct. I. The time interval between ovulation and egg recovery from the uterus in normal women. Fertil Steril 23:447, 1972
11. Pereda J, Croxatto HB: Ultraestructura del huevo humano al estado de siete células. Second Latin American Congress of Electron Microscopy, Ribeirao Preto, Brazil, December 1974
12. Burke CW, Geiselman CW: Exact anhydride epoxy percentage for electron microscopy embedding. J Ultrastruct Res 26:119, 1971
13. Zamboni L, Moore Smith D, Thompson RS: Migration of follicle cells through the zona pellucida and their sequestration by human oocytes in vitro. J Exp Zool 181:319, 1972
14. Pereda J, Croxatto HB: Unpublished data