FERTILIZATION AND CLEAVAGE OF THE HUMAN OVUM *in vitro*

A. I. Nikitin, M. P. Nikolaichuk,
and É. M. Kitaev

UDC 612.613-085.2

A method of cultivating and fertilizing human ova obtained from ovaries removed at operation is described. A special method of sampling active spermatozoa was used for fertilization. Zygote formation was observed 24-36 h after fertilization of the ripe ova, an embryo of the two-cell stage was formed after 36-40 h, and an embryo at the four-cell stage after 48 h. The number of blastomeres reached 8 after 60-64 h, and after approximately 100-120 h the embryos reached the morula stage. The results provide wide scope for the study of the physiology and pathology of early human embryonic development.
KEY WORDS: *human ovum; fertilization and development in vitro.*

The study of fertilization of the human ovum *in vitro* and cultivation of embryos at preimplantation stages of development is attracting ever-increasing interest at the present time. Such investigations owe their urgency to the need for a closer study of some aspects of preembryonic and early embryonic human development in order to shed light on the causes and mechanisms of origin of certain hereditary diseases, of early embryonic mortality, and also of various forms of sterility. Results obtained in experiments on mammals, moreover, cannot always be extrapolated to man.

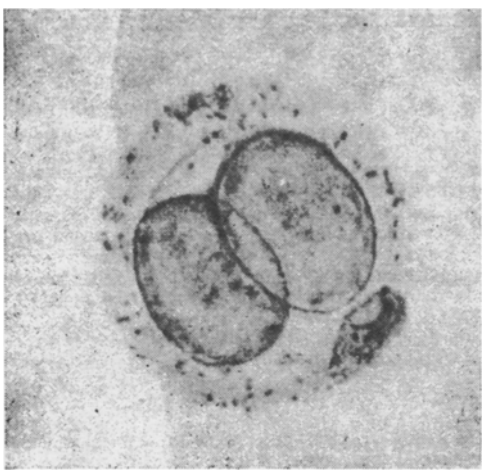

Fig. 1. Human embryo at the two-blastomere stage. Here and in Figs. 2 and 3: phase contrast, 120×.

However, despite its urgency, it is only recently that it has been studied on a large scale. The greatest success has been achieved by Edwards et al. [1, 2] and Seitz et al. [3], who have succeeded in developing fertilized human ova as far as the blastocyst stage. In the Soviet Union the problem of ripening and fertilization of human ova *in vitro* has been studied for several years in the Department of Obstetrics and Gynecology, Academy of Medical Sciences of the USSR [4, 5]. However, it must be admitted that, despite progress made, the undertaking of investigations of this type still involves considerable difficulties, mainly technical by nature, and the percentage of fertilized gametes and of zygotes in the stage of cleavage still remains low.

This paper gives the results of the writers' investigations into fertilization and cleavage processes in human ova *in vitro*.

EXPERIMENTAL METHOD

Ova were extracted from the follicles of ovaries removed for various indications (multiple cysts, inflammatory diseases, benign serous and pseudomucinous cysts, uterine fibromyoma, etc.).

Institute of Obstetrics and Gynecology, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. G. Baranov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 3, pp. 375-377, March, 1976. Original article submitted June 17, 1975.

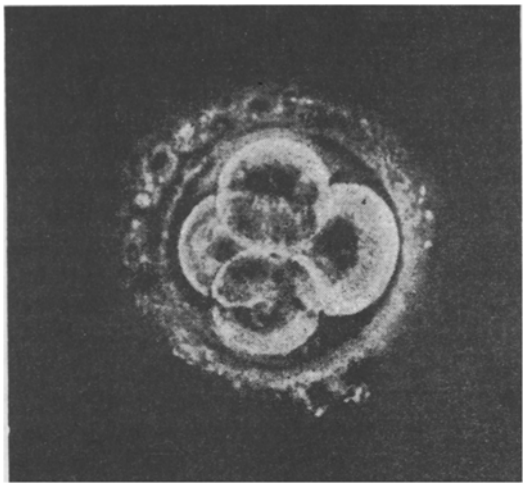

Fig. 2. Embryo at the four-cell stage.

Before the operation the women were given clomiphene and chorionic gonadotropin according to the scheme suggested by Steptoe and Edwards [6, 7]. The ova, removed from the ovaries, were grown for about 20 h in medium No. 199 with the addition of 15% bovine serum. The ova were then fertilized in modified Earle's solution by the method described previously [4].

Spermatozoa were treated as follows before fertilization. The fresh ejaculate was diluted with Earle's solution in the ratio of 1:6 and centrifuged. A concentrated suspension of spermatozoa was prepared from the residue in a fresh batch of Earle's solution and pipets filled with 0.3 ml of the same solution, to which follicular fluid was added, were immersed in it. In the course of 1 h at 37°C the spermatozoa "swam" into the medium and so rid themselves of seminal plasma and other impurities present in the seminal fluid. The "washed" spermatozoa were incubated for a further hour under the same conditions. Next, ova ready for fertilization were placed in a drop of the spermatozoal suspension measuring not more than 0.03 ml in volume. The concentration of spermatozoa was 10^4-10^5 cells/ml.

The ova were incubated with spermatozoa in a special chamber at 37°C in drops of medium under mineral oil in a mixture of air with 5% CO_2, and with periodic monitoring of the pH and osmolarity of the medium.

EXPERIMENTAL RESULTS

Altogether 179 ova were selected for fertilization. Most of the ova taken from the ovaries were at the stage of diakinesis or metaphase 1 of meiosis, as shown morphologically by disintegration of the germinal vesicle in these ova. The completion of the first maturation division, the appearance of the polar body and formation of the perivitaline space, were observed after about 20-24 h. By the time of fertilization most ova were still surrounded by a fair amount of follicular epithelium, although signs of its degeneration and of weakening of the connection between the granulosa cells and ova increased gradually. Spermatozoa concentrated around the ova that preserved their corona radiata were most active and retained their mobility longer than spermatozoa concentrated around cells which had lost their follicular epithelium. Although the role of the follicular epithelium in the hormonal regulation of energy homeostasis of maturation and fertilization processes in mammals has not been entirely settled, in all probability the granulosa cells help to create the conditions necessary for completion of the ripening of the ova *in vitro*, and in the process of fertilization they may have a positive effect on spermatozoa.

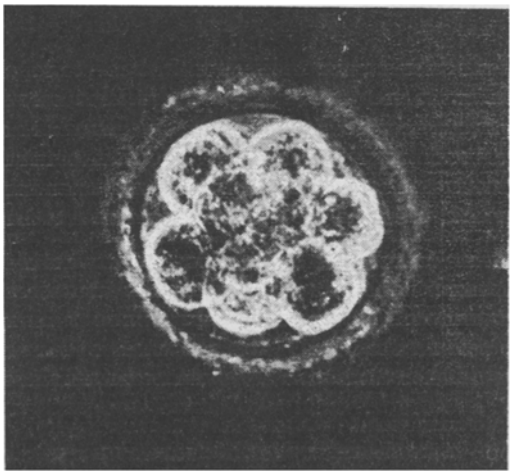

Fig. 3. Human embryo at the eight-blastomere stage.

From 12 to 15 h after fertilization pronuclei and the second polar body appeared, and the zygote began to form after 24-36 h. At this stage the fertilized ova were transplanted into Ham's medium [8] to which blood serum from women in the second phase of the menstrual cycle was added. During the next 36-40 h fusion of the pronuclei, formation of a zygote with a diploid set of chromosomes, and its di-

vision into two blastomeres took place (Fig. 1). The appearance of embryos at the stage of four blastomeres (Fig. 2) was observed 48 h after the beginning of fertilization, and after 60-64 h the formation of an eight-cell stage was observed (Fig. 3). Approximately after 100-120 h, morula formation was observed. Further growth of the morulas under the same conditions led to their gradual degeneration.

Of the 179 ova, 4 developed only as far as the pronucleus stage, 10 formed a zygote, 8 reached the two-blastomere stage, 6 developed to the four-cell stage, 2 to the eight-blastomere stage, and 2 cells formed a morula. Consequently, the total number of fertilized ova was 32 (17.8%).

In vitro, successful fertilization of human ova thus was obtained and conditions were created for the embryo to develop as far as the eight-blastomere stage; this is of fundamental importance for it is at that stage of development that the embryo enters the uterus, where it will grow until it is time to prepare for implantation [9]. Reproduction of these early stages of the embryo *in vitro* and the study of the necessary conditions for further growth of the embryo will permit the details of fertilization and cleavage of the ovum in women *in vivo* during the period of tubal development to be studied in detail and will provide an approach to the solution of the problem of transplantation of such embryos into women with various forms of sterility.

LITERATURE CITED

1. R. G. Edwards, B. D. Bavister, and P. C. Steptoe, Nature, 221, 632 (1969).
2. R. G. Edwards, Acta Endocrinol. (Copenhagen), 71, 131 (1972).
3. H. M. Seitz, G. Rocha, B. G. Brackett, et al., Fertil. and Steril., 22, 255 (1971).
4. M. A. Petrov-Maslakov, A. I. Nikitin, and M. P. Nikolaichuk, Byull. Éksperim. Biol. i Med., No. 12, 83 (1973).
5. A. I. Nikitin, Abstracts of Proceedings of the 5th All-Union Conference of Embryologists [in Russian], Moscow (1974), pp. 129-130.
6. P. C. Steptoe and R. G. Edwards, Lancet, 1, 683 (1970).
7. R. G. Edwards and P. C. Steptoe, Proc. Roy. Soc. Med., 67, 932 (1974).
8. R. G. Ham, Exp. Cell Res., 29, 515 (1963).
9. H. B. Croxatto, S. Diaz, B. Fuentealba, et al., Fertil. Steril., 23, 447 (1972).