FERTILIZATION OF HUMAN OVA IN VITRO

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A method of fertilizing and cultivating fertilized human ova in vitro is described.

The fertilization of human ova in vitro was first reported by Rock and Menkin [12, 14]. In isolated cases they succeeded in fertilizing an ovum and obtaining an embryo at the stage of 2 blastomeres. However, these experiments were not subsequently developed. The observations of Petrov [1, 2] on fertilization of human ova in vitro were not confirmed and were criticized [4]. Shettles [15-18] published a series of papers on fertilization of human ova in vitro and on the development of the embryo up to the blastula stage.

A special place is occupied by the paper by Petrucci [13] describing fertilization of ova in vitro and cultivation of a human embryo in vitro for 58 days. No later worker has ever succeeded in repeating his experiments. Recently some intensive research into the development of methods of ripening human oocytes in vivo, on their fertilization and development of the embryo in vitro has been undertaken by Edwards and his group [5-10] in Cambridge. These workers have succeeded in fertilizing an ovum in vitro and observing development of the embryo to the blastocyst stage.

Besides this group of workers, Jacobson et al. [11] have also described the successful fertilization of human ova in vitro.

Nevertheless, fertilization of human ova in vitro still remains a difficult problem and the percentage of fertilized gametes is very low. Nevertheless, the successful reproduction of this process in vitro is an essential preliminary to the solution of some important problems in the physiology and pathology of human reproduction.

In this paper the writers present their own observations on the fertilization of human ova in vitro.

EXPERIMENTAL METHOD

Ova were extracted from the follicles of ovaries removed at operation for various clinical conditions (polycystic disease, ovarian cysts and tumors, and so on) and were cultivated in vitro until they reached the stage of readiness for fertilization. The method of cultivation was described previously [3]. Oocytes which have passed the first maturation division are able to be fertilized. A morphological sign of the completion of this process is isolation of the first polar body (Fig. 1), i.e., the formation of a gamete with a haploid set of chromosomes. In vitro this process usually takes place within 30-40 h of the beginning of cultivation.

The medium in which the ova were kept and which had been used for maturation of the oocytes was then replaced by a fertilization medium including NaCl 8 g/liter, KCl 0.4 g/liter, $CaCl_2$ 0.2 g/liter, $MgCl_2$ 0.1 g/liter, NaH_2PO_4 0.1 g/liter, $NaHCO_3$ 3.0 g/liter, glucose 1 g/liter, sodium pyruvate 0.009 g/liter, and bovine serum 200 ml/liter; pH 7.6.

Before addition to the culture medium with the ova the spermatozoa were treated as follows. The fresh ejaculate was mixed with the above-mentioned medium (without serum) in the ratio of 1:6 and centrifuged twice. The spermatozoa were then incubated for 2 h at 37°C in the same medium to which a few drops

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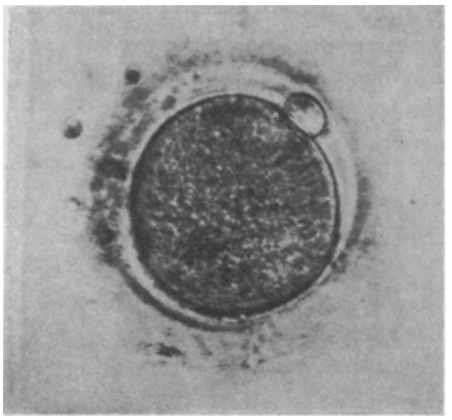

Fig. 1

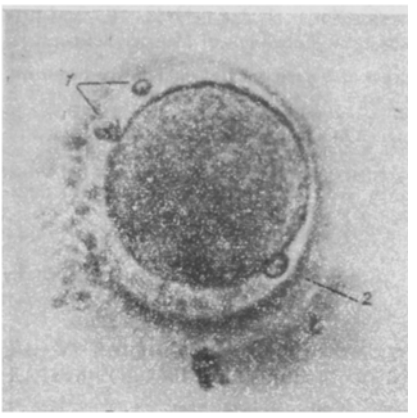

Fig. 2

Fig. 1. Living human ovum (gamete) extruding the first polar body after cultivation for 30 h in vitro. Here and in Figs. 2 and 3: phase contrast, 100 ×. Photomicrograph.

Fig. 2. Fertilized ovum extruding second polar body (2); first polar body (1) has also separated.

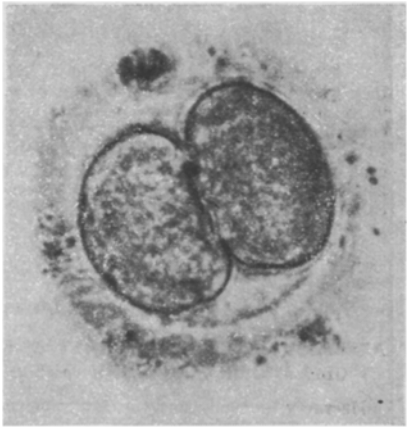

Fig. 3. Human embryo at the 2-blastomere stage.

of follicular fluid had been added. At the end of this period the spermatozoa were added to the culture medium with the ova in a concentration of 0.5×10^6/ml.

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

EXPERIMENTAL RESULTS

During observations on the process of fertilization no purposive movements of the spermatozoa relative to the ovum could be detected. Their movements were irregular and contact with the ovum took place by accident. However, once in contact with the outer surface of the zona pellucida, the spermatozoa adhered firmly to it, so that in the course of 1-2 h the overwhelming majority of them were concentrated on the outer surface of the ova. Penetration of the spermatozoa through the zona pellucida into the perivitelline space took place after about 5 h, and in the course of 7-15 h the second polar body was extruded (Fig. 2) and the pronuclei were formed.

The fertilized ova were transferred into medium No. 199 containing 15% bovine serum 15-20 h after addition of the spermatozoa to the medium.

Zygote formation, according to these observations, took place after 24-30 h with separation into two blastomeres (Fig. 3) after 36-40 h and the formation of the 3-cell stage of the embryo 48-52 h after the beginning of fertilization. In preparations fixed and stained with orcein a diploid set of chromosomes was already present in the mitotic phase of the zygote; in the blastomeres interphase nuclei could be seen.

Of 490 oocytes which were fertilized, 30 (6.1%) were at one of the stages described above.

The necessity for distinguishing unfertilized, degenerating gametes from cleaving, fertilized ova must be mentioned. Degenerative changes in the ova follow a special course with the appearance of fragmentation of the cytoplasm which resembles its separation into blastomeres. However, the great irregularity in size of the fragments, the asynchronous character of the "cleavage," and the shortening of the time of formation of the "parvocellular" structures must be emphasized. When the fixed preparations were stained, no nuclear structures could be detected in the fragments of cytoplasm of the degenerating unfertilized gametes.

The results confirm that fertilization of human ova can be reproduced in vitro, and they offer prospects for the closer study of the intimate processes of conception in man. They permit a comprehensive investigation of the early (preimplantation) stages of development of the embryo, which will provide the answer to many important problems in the physiology and pathology of human reproduction. Success in the in-vitro cultivation of embryos in the early stages will also contribute toward the solution of the problem of transplantation of human embryos.

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