DEVELOPMENT OF GONADS OF 7-11-WEEK HUMAN FETUSES IN ORGAN CULTURE

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A simplified method of organ culture of human gonads is suggested. Altogether 190 gonads from 7-11-week embryos were grown on Millipore filters in penicillin flasks. During the first week of culture areas of central necrosis were observed in both the ovaries and the testes, but during subsequent cultivation these disappeared in some of the explants. Progressive development of germinative cells took place in the ovaries of 9-11-week embryos as far as the pachytene stage, whereas development in the ovaries of 7-9 week embryos did not reach the leptotene stage. Germinative cells during cultivation of the testes did not progress beyond the spermatogonium stage.

KEY WORDS: human oogenesis; culture of human gonads; prophase of meiosis.

Some of the problems that arise during the study of human oogenesis and spermatogenesis can be investigated by the method of organ culture. Only a few attempts to grow human gonads in culture have been described [3, 4, 6]. For organ culture the authors cited used ovaries of fetuses at the late stages of development, in which the germinative cells were mainly in various stages of prophase of meiosis.

In the experiments described below ovaries from human fetuses in the early stages of development were used for organ culture. Besides oogonia, preleptotene stages of oocytes are also found in the ovaries at this period.

The technique of cultivating mammalian and human gonads has been described previously [3, 4, 6-8, 13]. Having tested several methods of organ culture, it was decided to use a variant developed in the writer's laboratory and combining the "platform" [12] and "raft" [5] methods into one. The suggested modification of gonad culture is technically simple and gives reproducible results.

EXPERIMENTAL METHOD

Experiments were carried out on 190 gonads (127 ovaries and 63 testes) from human fetuses at the 7th-11th week of development (material obtained from therapeutic abortions). The fetuses were sent to the laboratory in Hanks' solution. The gonads were isolated under sterile conditions and washed in several batches of Eagle's medium.

The organs were cultivated on Rufs Millipore filters (pore diameter 1.2-2.5 μ, dimensions of filter $3 \times 8 mm^2$, sterilized in 96° ethanol for 15 min and dried under bactericidal lamps). The filters with gonads from one embryo (ovaries from fetuses aged 10-11 weeks or older are cultivated in fragments) were placed in the central part of the bottom (with a convex surface) of a penicillin flask. Nutrient medium of the following composition was then poured into each flask: Eagle's medium (with the addition of glutamic acid) 85%, bovine serum 15%, penicillin in a final concentration of 100-150 units/ml. The volume of culture fluid in the flask was 0.3-0.5 ml; the filter must remain soaked with medium and the gonads above it in the gaseous phase. The flasks were tightly sealed with No. 14.5 rubber stoppers, placed in a rack, and incubated at 34° C [9]. The medium was changed every third or fourth day. The subsequent course of culture showed

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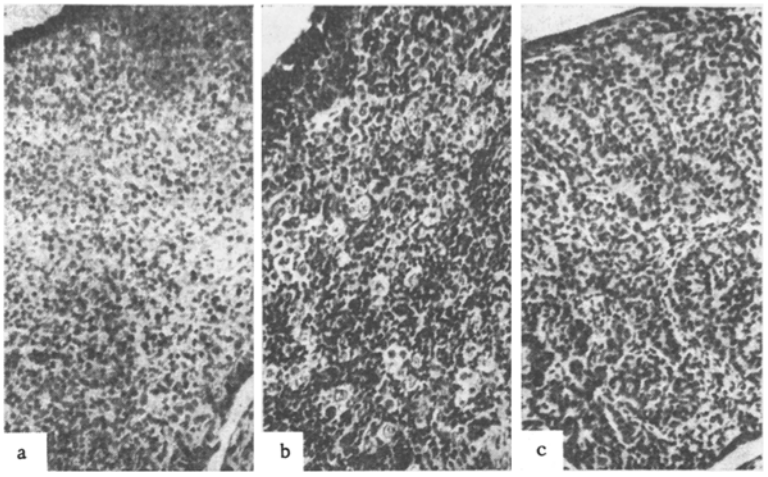

Fig. 1. General view of gonads after three weeks in culture: a) ovary of 7-8 week embryo; b) ovary of 10-11-week fetus; c) testis of 8-week embryo. Mayer's hematoxylin, 200 ×.

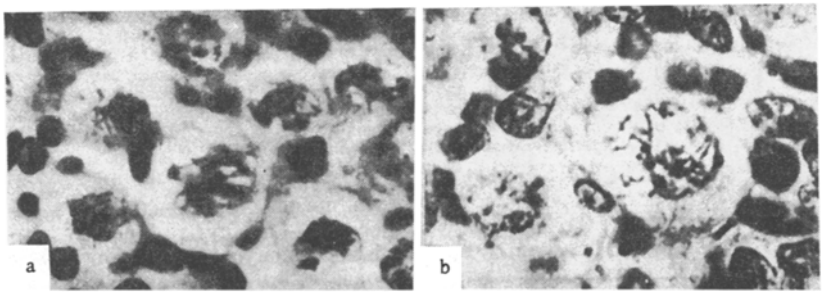

Fig. 2. Stages of prophase of meiosis in ovary of 10-11-week fetus after three weeks in culture: a) zygotene stage; b) pachytene stage. Mayer's hematoxylin, 900 ×.

that the volume of air present in the flask and the quantity of carbon dioxide excreted by the tissue during metabolism were adequate for metabolic processes for several days. When the medium was changed, the gaseous phase was renewed.

After the end of cultivation the gonads were fixed in Carnoy's fluid, and histological sections cut to a thickness of 5 μ were stained with Mayer's hematoxylin.

EXPERIMENTAL RESULTS

The gonads from the human fetuses were cultivated for periods ranging from 1 day to 7 weeks. During the first weeks 13 ovaries and 9 testes were fixed at different times. Analysis of this material showed that the surface epithelium and part of the tissue of the testis or ovary lying next to the epithelium were preserved in the gonads of both sexes, but in the central part of the organs foci of necrosis were observed. Mitotic division continued in the preserved cells. Oogonia and oocytes in preleptotene stages were observed in the ovaries. Central necrosis also was described by Gaillard [6]. This worker explained the absence of necrosis in some explants on subsequent cultivation by regneration of the organ and the development of sex cells from the primitive epithelium, which is not in accordance with modern views [1, 2]. No attempt was made in the present investigation to study changes in the explants in the early periods of cultivation. This problem requires special investigation.

Twelve ovaries and 10 testes were cultivated for two weeks. No necrotic changes were found in 70% of the ovaries and 83% of the testes. Oogonia and oocytes in the preleptotene stages were found in ovaries from 7-9-week embryos; a few oocytes in the leptotene stages and solitary cells at the pachytene stage of

prophase of meiosis were found in ovaries from 9-11-week fetuses. Mitotic cell division continued in the testes.

Twenty-six ovaries and 15 testes were cultivated for three weeks. No necrotic changes were found in 58% of the ovaries and 67% of the testes. Many of the cells in the ovaries from 7-9-week embryos were dividing by mitosis, but oocytes were found only in preleptotene stages. Meanwhile, besides mitotically dividing oogonia, the ovaries of 9-11-week fetuses contained many oocytes in the leptotene, zygotene, and pachytene stages of the prophase of meiosis (Figs. 1 and 2).

Thirty-six ovaries and 25 testes were cultivated for four weeks. Characteristic structures of the ovaries were found in 84% and of the testes in 64% of cases. By this time the organs in culture were reduced in size, especially the ovaries. The ovaries of the 7-9-week embryos contained predominantly follicular cells, with a few oogonia and single oocytes in preleptotene stages. Ovaries of the 9-11-week fetuses also contained chiefly follicular cells; a few oogonia and oocytes in various stages of preprophase and prophase of meiosis also were seen.

After cultivation of the gonads (six ovaries and four testes) for a longer time (5, 6, and 7 weeks) no further differentiation of the ovarian cells occurred, and in the testis the germinative cells did not start to undergo meiosis.

Examination of 30 control specimens of ovaries not grown in culture showed that single oocytes at the leptotene stage only begin to appear in ovaries of 10-11-week fetuses. According to Ohno et al. [10], oocytes at the zygotene stage appear in 12-week fetuses, and at the pachytene stage in 16-week fetuses. Under the conditions of organ culture, oocytes at the zygotene and pachytene stages were observed after cultivation of the ovaries of 9-11-week fetuses for 3-4 weeks. This indicates that the development of the germinative cells progress at about the same rate under these conditions of culture as in vivo. However, the pachytene stage was the last stage of prophase of meiosis to be observed.

The stages of prophase of meiosis were observed in oocytes only when ovaries from 9-11-week fetuses were cultivated, and they were not observed after cultivation of ovaries from 7-9-week embryos. Accordingly, it can be postulated that the important factor for successful development of ovarian germinative cells in vitro is the degree of development of the ovary in vivo before cultivation begins. These results are in agreement with those obtained by Martinovitch [8], who cultivated gonads of embryonic and newborn mice and rats. During cultivation of ovaries from 16-day mouse embryos (at this period of development, according to Peters and Crone [11], many oocytes were at the leptotene, zygotene, and pachytene stages), oocytes in the dictyotene stage and the formation of primary follicles could be observed. However, this development did not progress further, and after the 30th day of cultivation the organ degenerated. At the same time, when ovaries from newborn mice and rats were cultivated (the oocytes were in the dictyotene stage and were surrounded by follicular cells), growth of follicles and typical maturation divisions were observed. In the investigation by Gaillard [6], maturation divisions also were observed during the cultivation of ovaries from human fetuses in the later stages of development, and Blandau [4] described the formation of a primary follicle after cultivation of fragments of ovaries from human fetuses for 79 days.

The results obtained by cultivation of ovaries from human fetuses at the earlier stages of development, in which oocytes developed as far as the pachytene stage, evidently depend not so much on the method of cultivation as on the initial stage of development of the ovary in vivo before cultivation.

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