# HUMAN OOGENESIS STUDIED IN ORGAN CULTURE

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The degree of development of the germinative cells of the ovaries of human fetuses growing in organ culture and of control ovaries from fetuses of "equivalent" age was compared. Germinative cells of fetuses at 8-9 weeks of development and older were able to enter prophase of meiosis under organ culture conditions; in explants of ovaries of 7- to 8-week fetuses only solitary oocytes reached the leptotene stage. The appearance of oocytes at the leptotene, zygotene, and pachytene stages occurred at the same times in the culture as *in vivo*. However, the percentage of the cells of these stages in the explant as a rule was lower than in the control ovaries. After the addition of radioactive thymidine-$^3$H into the culture, labeled oocytes at the zygotene stage appeared in 4 days and at the pachytene stage in 14 days.

KEY WORDS: human oogenesis; cultivation of human ovaries; prophase of meiosis.

Organ culture of mammalian gonads has recently been used on a wide scale. It has been shown [7, 11, 15], for instance, that explantation of the ovaries of mammalian (mouse, sheep, hamster) fetuses, once a certain critical period before the beginning of meiosis has been reached, is accompanied by the typical development of sex cells. Under these circumstances, in culture medium not containing hormones the sex cells enter the prophase of meiosis at the proper time and pass through the successive stages of prophase at a rate comparable with the rate of their development *in vivo*. During cultivation of ovaries taken before the critical period, the number of sex cells in the explant decreases sharply and only a few cells enter the prophase of meiosis. Addition of gonadotropic hormones has no effect on the initiation of oogenesis in culture or on the passage of the oocytes through the early stages of prophase of meiosis [6, 7]. However, these hormones are essential for subsequent growth of the oocytes, for follicle formation, for maturation of the follicles, and also for normal development of the ovarian stroma [5, 8].

Despite the fact that on addition of hormones under organ culture conditions completely normal development of the sex cells from the oogonium stage to the ovulating ovum has not yet been obtained, by explanting the ovaries of fetuses of different ages it is possible to observe the various stages of oogenesis in culture in practically the same form as they occur *in vivo*.

This makes it possible to use the organ culture method for determining the temporal parameters of preprophase and prophase of meiosis in man.

Investigations of this sort, conducted on cultures of adult human testes [10, 12], have shown that the duration of the corresponding stages of spermatogenesis in culture is similar to their duration *in vivo* [9].

Baker and Neal [6], who cultivated the ovaries of 9- to 15-week human fetuses by a method developed previously [4], observed progress in the development of germinative cells incorporating thymidine-$^3$H at the beginning of the experiment as far as the stage of zygotene (10th-12th day), pachytene (20th day), and diplotene (28th day).

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TABLE 1. Changes in Numbers of Oogonia and Oocytes during Development of Ovaries *in vivo* and *in vitro*

| Number of ovaries | Age of fetus, weeks | Period of cultivation, weeks | Stage of development of germinative cells, % | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | oogonia | oocytes (total) | oocytes in prophase of meiosis | | | atresia |
| | | | | | leptotene | zygotene | pachytene | |
| 7 | 7—8 (control) | — | 76 | 24 | — | — | — | 0,6 |
| 9 | 8—9 (control) | — | 66 | 34 | — | — | — | 0,7 |
| 7 | 9—10 (control) | — | 50 | 50 | 0,02 | — | — | 0,2 |
| 10 | 7—8 | 2 | 80 | 18 | 0,08 | — | — | 1,6 |
| 7 | 10—11 (control) | — | 41 | 59 | 0,7 | — | — | 0,6 |
| 8 | 8—9 | 2 | 70 | 29 | 0,2 | — | — | 0,5 |
| 1 | 11—12 (control) | — | 15 | 82 | 3,5 | 8,8 | 0,3 | 3,0 |
| 5 | 9—10 | 2 | 55 | 40 | 3,9 | 3,2 | 0,6 | 4,0 |
| 8 | 8—9 | 3 | 35 | 64 | 0,8 | 0,2 | 0,1 | 2,0 |
| 0 | No control | | | | | | | |
| 1 | 10—11 | 2 | 28 | 68 | 4,1 | 12,5 | 3,6 | 4,0 |
| 6 | 9—10 | 3 | 29 | 70 | 2,3 | 1,1 | 0,1 | 1,0 |

The object of this investigation was to discover the critical time in the development of human ovaries when the germinative cells become capable of entering into prophase of meiosis *in vitro*, to compare the development of the germinative cells *in vivo* and *in vitro*, and to determine several temporal parameters of the preprophase and prophase of meiosis.

## EXPERIMENTAL METHOD

Human fetuses at the 7th-12th weeks of gestation (material from therapeutic abortions) were investigated. The age of the fetuses was determined from the crown—rump length, the length of the foot, the weight of the kidney and adrenal, and certain other criteria [1]. Some of the ovaries were fixed immediately after arrival of the fetuses (control), whereas the rest were used as explants. The ovaries were cultivated by the method developed in the laboratory [3] for 1-5 weeks.

Thymidine-$^3$H (specific activity 1.4 Ci/mmole) was added to the culture in the 2nd-3rd week of growth. The final concentration of labeled thymidine was 5-10 μCi/ml medium. After 1-1.5 h the thymidine was washed out by three or four changes of medium and cultivation continued. Some of the explants were fixed immediately after removal of the thymidine. The material was fixed in Carnoy's and Bouin's fluids and embedded in paraffin wax. Sections 5 μ in thickness were dewaxed and then coated with liquid type M emulsion diluted 1:3, exposed for 7-10 days at 4°C, and developed with amidol developer. The sections were stained with Carazzi's hematoxylin.

During analysis of the sections 500-1000 germinative cells were counted, distinguishing between oogonia and oocytes in the successive stages of preprophase and prophase of meiosis. Preprophase oocytes were divided into five stages depending on the degree of condensation or decondensation of the chromatin [2, 13, 14].

## EXPERIMENTAL RESULTS

The development of the ovaries in culture depended on the initial age of the fetus [3]. During cultivation of the ovaries of 7- to 8-week fetuses, progressive development of the sex cells was not observed: The ratio between oogonia and oocytes at the preleptotene stages of development was virtually unchanged. The total number of germinative cells decreased, as also did the size of the explants. However, after 2 weeks single oocytes were seen at the leptotene stage in some cultures. Some delay in development of the germinative cells also was observed in cultures of ovaries from fetuses taken at later stages of gestation. For in-

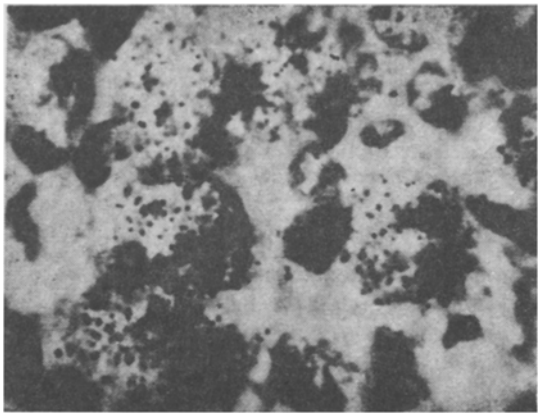

Fig. 1. Labeled oocytes in stage V of preleptotene — premeiotic DNA synthesis.

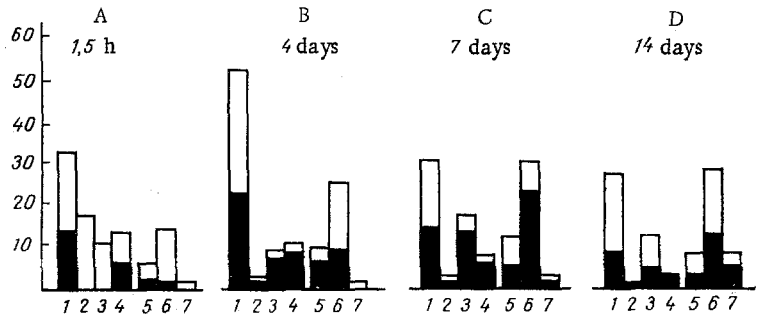


Fig. 2. Changes in relative numbers of germinative cells in successive stages of development and progress of labeled cells through stages of prophase of meiosis in cultures of ovaries from 11- to 12-week fetuses. Ordinate, number of cells in a given stage in % of total number of cells counted; abscissa, stages of development of germinative cells. 1) Oogonia; 2) preleptotene stages I and II; 3) preleptotene stages III and IV; 4) preleptotene stage V; 5) leptotene; 6) zygotene; 7) pachytene. Unshaded columns show unlabeled cells, black columns show labeled cells. A) 1.5 h; B) 4 days; C) 7 days; D) 14 days after pulse labeling of culture with thymidine-$^3$H.

stance, after 2 weeks in culture as a rule the percentage of oogonia in the explants was increased, and the percentage of oocytes in the preleptotene stages of development was correspondingly reduced. This was due at least partially to intensified division of the oogonia, for the percentage of dividing oogonia in the culture was 1.5-2 times greater than in the control. After 3 weeks, despite the preservation of a high level of mitotic activity, the fraction of oocytes was increased but the ratio between the numbers of oocytes and oogonia did not reach the control value. The percentage of oocytes entering into the prophase of meiosis also as a rule was lower than in the control ovaries of "equivalent" age (Table 1).

However, delay in the development of the germinative cells in explants of ovaries from fetuses at the 8th-12th week of gestation was purely quantitative in character: Stages of prophase of meiosis most advanced in development appeared at the same times as they would have appeared *in vivo* (Table 1).

From 1 to 1.5 h after addition of labeled thymidine to the culture the label was found chiefly in germinative cells in two stages: oogonia (premitotic synthesis) and oocytes at stage V of preleptotene, with despiralized prochromosomes (premeiotic synthesis of DNA; Fig. 1). Under these circumstances the mean number of grains of silver above the labeled oogonia and oocytes was about equal. This points to a similar duration of premitotic and premeiotic periods of DNA synthesis.

After 12 h many labeled oocytes in premeiotic stages of development were found in the cultures, and after 42 h they accounted for 80% of all labeled germinative cells. The percentage of labeled oogonia fell from 70 to 10. Consequently, the majority of oogonia incorporating labeled thymidine were in the last premitotic S period at the time of contact with thymidine. Having completed DNA synthesis, and when passing through the $G_2$ period and mitosis, these cells (or more probably, their progeny) become oocytes of the preleptotene period, at the end of which premeiotic DNA synthesis takes place.

Progress of the labeled germinative cells through the stages of prophase of meiosis took place most demonstratively in cultured fragments of ovaries from an 11- to 12-week fetus. Thymidine-$^3$H was added to the culture for 1.5 h on the seventh day of cultivation. Explants were fixed 1.5 h and 4, 7, and 14 days later (Fig. 2). Labeled thymidine was taken up by oogonia, oocytes of preleptotene stage V, and a few oocytes at the leptotene and zygotene stages. After 4 days oocytes which were in the premeiotic S period at the beginning of the experiment had progressed to the zygotene stage and the number of labeled cells in that stage was increased from 1 to 9%. On the seventh day the number of labeled oocytes at the zygotene stage was more than three times greater than the total number of oocytes which took up thymidine at the beginning of the experiment. It can be concluded from these observations that some oogonia which were in the last, premeiotic, S period at the beginning of the experiment had reached the zygotene stage by the seventh day. On the seventh day labeled oocytes at the pachytene stage appeared in the culture. Their number, like the total number of oocytes at the pachytene stage increased considerably until the 14th day.

It can be calculated approximately from these results that the pathway of development from the premeiotic S period to zygotene (the duration of the premeiotic $G_2$ period and leptotene) of the human oocyte takes 4 days. The duration of the zygotene stage in female meiosis is 10 days.

The experiments thus showed that, starting from the ninth week of fetal development, the germinative cells of human ovaries can enter into the prophase of meiosis in ovaries explanted in organ culture. The times of appearance of the early stages of prophase of meiosis (leptotene, zygotene, and pachytene) in explants of ovaries from 8- to 12-week fetuses are the same as the times of their appearance *in vivo*, but by the number of cells entering into the corresponding stage of development at the right time, the development of the explants lags behind development of the ovaries *in vivo*. The duration of the initial stages of prophase of female human meiosis is comparable with the duration of these stages in male meiosis, although the former takes place in the antenatal period of human development, the latter starting from the period of sexual maturation.

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