COMPARISON OF THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN POPULATIONS OF RAT OOCYTES MATURING in vitro AND OVULATING NORMALLY

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Investigations aimed at comparing the frequency of chromosomal aberrations in a population of "follicular" (taken from hollow follicles) and ovulating oocytes are almost nonexistent in the literature. Only in one publication [9] is evidence given to show that in CBA mice, of average reproductive age, the frequency of heteroploidy in a population of "follicular" oocytes is about 12%, whereas the level of chromosomal anomalies in a population of ovulating oocytes from the same animals does not exceed 2.6% [2]. It can be tentatively suggested that these differences, determining the rate of elimination of the gametes in ontogeny, and also the frequency of formation of subnormal gametes, acquire particular importance during hormonal stimulation of ovulation in women complaining of sterility. During stimulation of follicle production, the number of subnormal gametes is increased [3, 10]. The genesis of their formation has been discussed from two aspects: 1) during administration of exogenous hormones conditions are created for a disturbance of synchronized maturation and ovulation of the gametes; 2) hormonal stimulation makes possible the ovulation of oocytes which, under physiological conditions, would undergo atresia. Accordingly, the study of the frequency of chromosomal aberrations in a population of "follicular" oocytes is of urgent importance.

In this investigation the state of "follicular" rat oocytes was studied depending on the stage of the cycle with respect to the frequency of starting meiosis after culture for 42-46 h, the number of degenerating oocytes, the frequency of spontaneous cleavage, and also the frequency and character of the chromosomal aberrations. "Follicular" and ovulating oocytes were compared with respect to the criterion of chromosomal aberrations.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats aged 5-6 months and weighing 140-160 g. Altogether 66 rats were used. Oocytes were removed from follicles, visible to the naked eye, in rats at different stages of the cycle. Oocytes were cultured by the usual laboratory method in Ham's F10 medium with the addition of 10-15% calf embryonic serum at 36.8°C in an atmosphere of 5% CO_2 for 42-46 h. Ovulating oocytes were removed from the oviducts of rats in the stage of estrus, by puncture of the ampulla of the oviducts. Total preparations were obtained by Tarkowski's method [11]. The frequency of chromosomal aberrations was calculated relative to the number of cells with no morphological signs of degeneration of the chromosomes after culture.

EXPERIMENTAL RESULTS

Depending on the stage of the cycle, from 12-15 to 30 cells could be removed from the ovaries. The main results of the investigation are given in Table 1. The frequency of resumption of meiosis in the population of cultured oocytes averaged 69%. The largest number of cells resuming meiosis was observed at the stages of metestrus and diestrus. At the metestrus stage a considerable increase was observed in the number of oocytes with morphological signs of degeneration of the chromosomes (55.9 ± 5.4% compared with 31.8-35.9% at the diestrus-proestrus-estrus stages). At the proestrus stage oocytes undergoing spontaneous cleavage were observed; at the estrus stage their number reached 8.5%.

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TABLE 1. Frequency of Chromosomal Aberrations in a Population of "Follicular" Rat Oocytes Depending on Stage of the Cycle

Stages of cycle	No. of oocytes	Frequency of resumption of meiosis	No. of degenerating oocytes	Frequency of spontaneous cleavage	No. of oocytes suitable for analysis (metaphase II)	Chromosomal aberrations, %		
						aneuploidy	polyploidy	fragments
		%						
1. Diestrus	160	81,2±3,1	35,6±3,8	—	44,5±6,6	—	1,65	—
2. Proestrus	253	75,1±2,7	35,9±3,0	0,79	37,5±5,7	1,10	1,10	—
3. Estrus	593	61,1±2,0	31,8±1,9	8,57	25,8±4,5	1,87	3,75	—
4. Metestrus	84	80,9±4,3	55,9±5,4	3,57	22,6±10,63	3,03*	7,57*	1,2
		P_1 and P_3 $_3<0,05$ $_4<0,05$	P_4 and $_{1-3}<0,05$		P_1 and $_3<0,05$			
Total . . .	1090	69,0	68,0		32,0	8,2		

*Oocytes showing signs of degeneration of chromosomes, which were disregarded when the average frequency of chromosomal aberrations was calculated in the population of "follicular" oocytes.

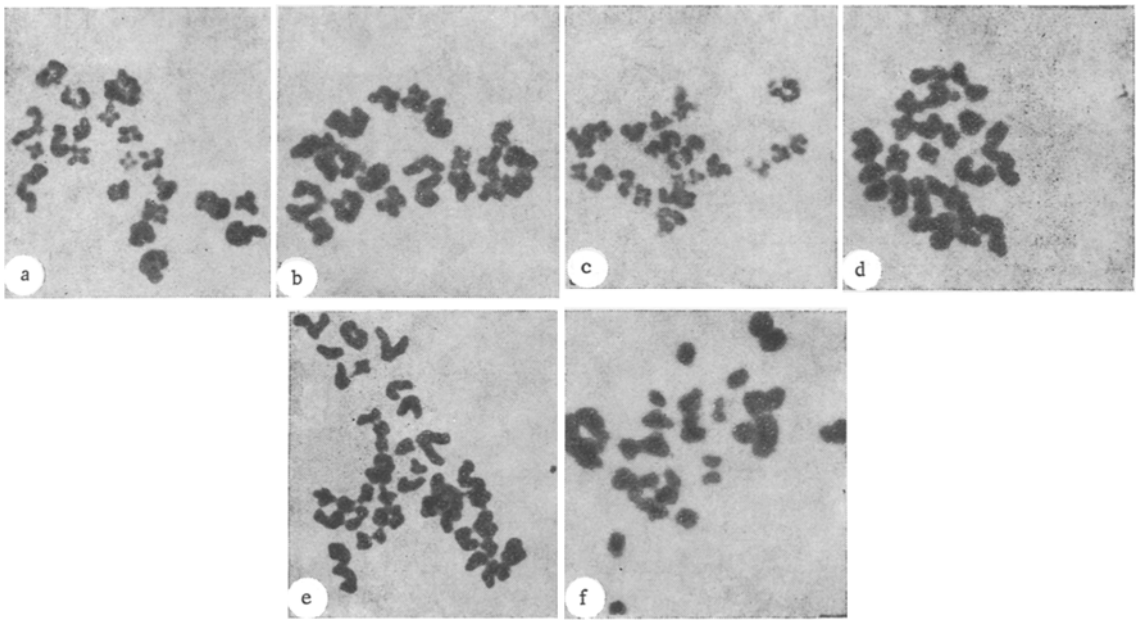

Fig. 1. Chromosomes of rat oocyte at metaphase stage of division II of meiosis (42 h of culture). a) Normal set of chromosomes (n=21); b) hypoploid number of chromosomes (n=18); c) hyperploid number of chromosomes (n=22); d) presence of a chromosome fragment (arrow); e) degeneration of chromosomes at metaphase stage of division II of meiosis; f) diploid set of chromosomes arising in rat oocyte as a result of block to anaphase of division I.

Chromosomal analysis of the culture oocytes showed that the largest number of oocytes with an unchanged structure (Fig. 1a) was observed at the diestrus stage (44.5 ± 6.6%), at which the "follicular" oocytes also were characterized by the lowest frequency of chromosomal aberrations. Meanwhile, in the subsequent stages of the cycle, an increase was observed in the frequency of chromosomal aberrations (Fig. 1b-e) which reached a maximum at the stage of metestrus. The mean frequency of chromosomal aberrations in the population of "follicular" oocytes (disregarding the stages of the cycle) was 8.2%.

The study of the population of ovulating oocytes revealed 16 of 442 cells with numerical aberrations: four (0.9%) with a hyperploid set of chromosomes (n>21) and 12 (2.7%) hypoploid cells (n<21). Depending on the number of hyperploid cells, the mean frequency of chromosomal aberrations in the population of ovulating oocytes was thus 1.8%.

The results are evidence that the populations of "follicular" and ovulating oocytes differ significantly in their level of chromosomal aberrations. Analysis of oocytes taken from follicles showed that their state changed considerably in the course of the cycle; this evidently reflects the special features of follicle production that depend on changes in the hormonal status. In rats during the period of preovulatory discharge of gonadotrophins the main mass of antral follicles undergoes atresia [7]. The oocytes of such follicles, as has been shown, are frequently eliminated and meiosis is resumed [1, 5, 8], and it is probably this which causes the increase in the frequency of reinitiation of meiosis in metestrus and diestrus, the increase in the number of aberrant oocytes or oocytes degenerating in the course of culture, removed during metestrus, and also the number of ova undergoing spontaneous cleavage in proestrus and estrus. During degeneration changes in the apparatus of division and cortical granules are among the earliest disturbances taking place in the oocyte [4]. Predominance of numerical aberrations (on account of incorrect separation of the chromosomes in meiosis) is evidence that the majority of chromosomal anomalies in the population of "follicular" oocytes maturing in vitro was associated with injury of this type to the gametes. Most oocytes carrying chromosomal anomalies thus evidently are sex cells which have been eliminated during gametogenesis. Since it has been shown that in some strains of mice the number of aberrant gametes is increased as a result of hormonal stimulation of follicle production [6], it must be concluded that the data described above much be taken into account when hormonal stimulation of ovulation is used in the treatment of women for sterility.

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