SPONTANEOUS CHROMOSOMAL ABERRATIONS IN OOGENESIS OF LABORATORY RATS

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Of 861 oocytes with chromosomes suitable for karyotyping and accurate counting, 797 (92.7%) had the normal number of chromosomes, 64 (7.5%) had aneuploidy, four (0.4%) hyperploidy, and in the remaining 60 there was a deficiency of chromosomes (hypoploidy). Hypoploidy was often caused by artefacts. The percentage of spontaneous aneuploidy in rat oogenesis is evidently about 0.8, much lower than the percentage of spontaneous aneuploidy in mouse oogenesis.

KEY WORDS: *rat oocytes; aneuploidy; karyotyping; nonseparation of chromosomes.*

An important and urgent task in cytogenetics is to study the causes of appearance of aneuploid embryos, i.e., spontaneous abortions, embryonic death, and congenital malformations [3]. An effective approach to this complex problem is the study of nonseparation of chromosomes in the gametes not only of man, but also of laboratory mammals. However, whereas some data have already been obtained on nonseparation of chromosomes in meiosis during spermatogenesis in these animals [12], the frequency of spontaneous nonseparation of the chromosomes in oogenesis has been inadequately studied, and moreover, only incomplete information on nonseparation of chromosomes in oogenesis has been published for laboratory mice [10], whereas in rats this problem has evidently not been studied at all.

It was therefore decided to study the frequency of spontaneous nonseparation of chromosomes in the oogenesis of laboratory rats.

EXPERIMENTAL METHOD

Young albino rats weighing 80-100 g from the Rappolovo nursery in which ovulation was induced by hormones were used. The female rats initially received an injection of 30 units serum gonadotropin, followed by chorionic gonadotropin in a dose of 10 units 56 h later. The females were killed 13-17 h after injection of the second hormone, the oviducts were removed, the ampulla of the oviduct was torn with a dissecting needle, and the oocytes were removed together with the follicular cells and surrounding mucus. The coagulum was transferred to medium No. 199 to which hyaluronidase was added [5]. When the oocytes were freed from the follicular cells they were transferred by a micropipet into a fresh portion of medium No. 199, then placed in a hypotonic solution (0.9% sodium citrate or 0.56% potassium chloride), and chromosome preparations were then made by Tarkowski's method [13]. The preparations were stained either with lactacetoorcein or by Giemsa's method (as described in [9]).

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TABLE 1. Spontaneous Chromosomal Aberrations in Second Metaphase of Rat Oocytes (84 rats; M ± m)

Total number of oocytes ovulating	Number fixed by Tarkowski's method	Number karyotyped	Normal karyotype (21 chromosomes)		Number of aneuploid oocytes							
					total	with 19 chromosomes		with 20 chromosomes		with 22 chromosomes	
			abs.	%		abs.	%	abs.	%	abs.	%
3106	2335	861	797	92,5±0,89	64	5	0,63±0,28	55	6,96±0,90	4	0,4±0,21

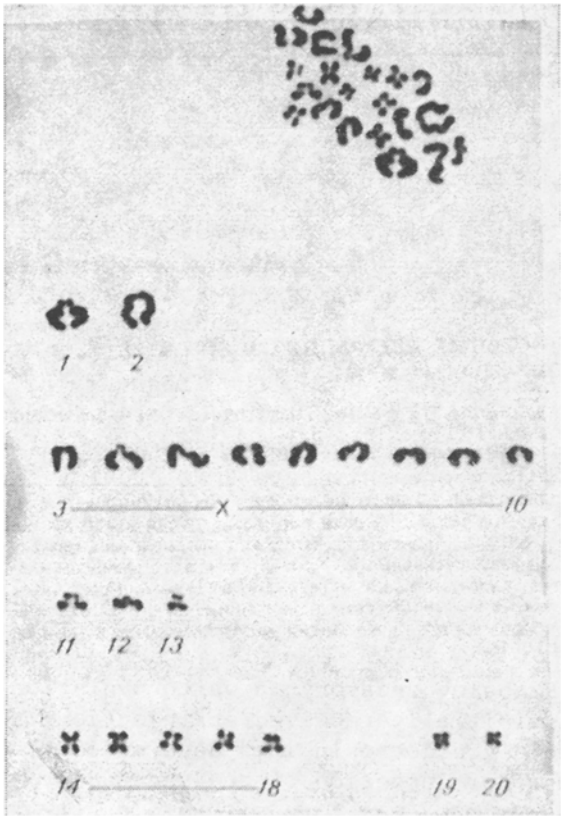

Fig. 1. Normal karyotype of rat oocyte.

EXPERIMENTAL RESULTS

Of 2,335 oocytes, only in 861 were the chromosomes suitable for karyotyping and accurate counting (Table 1); in the other cases the oocytes were at an unsuitable stage for their chromosomes were heaped together. Oocytes were investigated 13-18 h after injection of chorionic gonadotropin, when they were at the metaphase stage of the second maturation division. This corresponds with data in the literature on the chronology of maturation of rat oocytes [8]. By dealing with metaphases of the second maturation division it was possible to count the number of chromosomes and to compare the karyotypes in those cases when this number was abnormal. It will be recalled that for rats 2n = 42, i.e., under normal conditions in metaphases of the second maturation division there should be 21 chromosomes. In 92.5% of oocytes (797 of 861) the number of chromosomes was normal (Fig. 1), whereas in 7.5% of cases (in 64 oocytes) aneuploidy was present. Characteristically hyperploidy, i.e., 22 chromosomes, was found in only four oocytes (in 0.4% of cases), whereas a deficiency of chromosomes (hypoploidy) was observed in 60 oocytes.

Although the possibility that the higher frequency of hypoploidy than of hyperploidy might be due to selective elimination of hyperploid sets in the composition of the polar body [10] cannot be completely ruled out, it seems unlikely. It is more logical to suggest that loss of chromosomes took place during preparation of the specimens, i.e., that a large proportion of the hypoploid oocytes was the result of artefacts. It is difficult to accept that during preparation of the specimen an extra chromosome could be added, i.e., the frequency of hyperploid metaphases of the second maturation division evidently reflects the true picture. Evidently if the chromosomes failed to separate in meiosis, equal numbers of hypoploid and hyperploid gametes should be formed. If this is so, the frequency of hypoploid and hyperploid oocytes ought to be the same, i.e., the total frequency of nonseparation of chromosomes in rat oogenesis is 0.8%.

Karyotyping of four hyperploid metaphases showed that in one case the chromosome

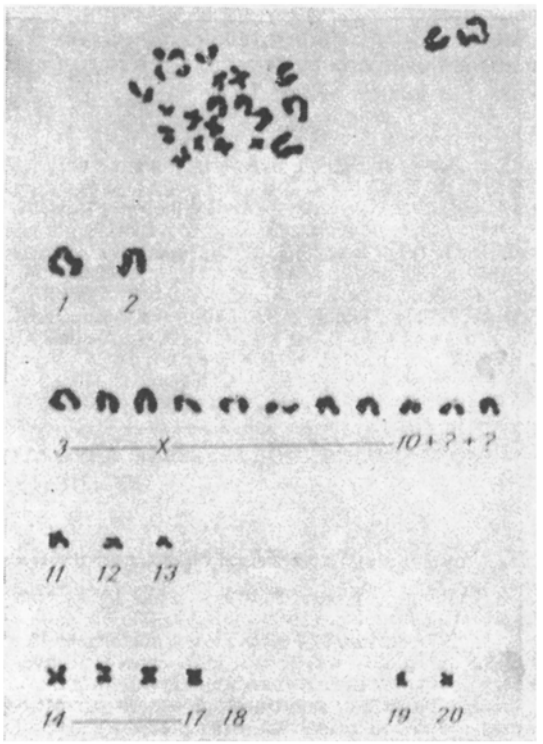

Fig. 2

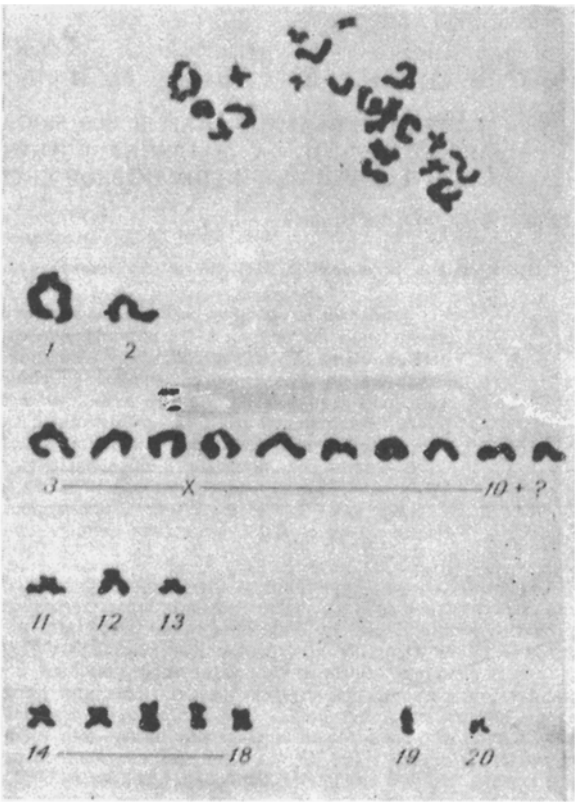

Fig. 3

Fig. 2. Aneuploid karyotype of rat oocyte. Hyperploidy. Two extra chromosomes in group 3-X-10. Absence of metacentric in group 14-18.

Fig. 3. Aneuploid karyotype of rat oocyte. Hyperploidy. Extra chromosome in group 3-X-10.

set had two additional acrocentric chromosomes (Fig. 2) in the 3-X-10 group and absence of the metacentric chromosome in the 14-18 group, whereas in three cases an extra chromosome was found in the 3-X-10 group (Fig. 3). Since nonseparation of the X-chromosome takes place more frequently that nonseparation of autosomes [11], this suggests that in three cases the oocytes contained, not one, but two X-chromosomes. This hypothesis requires confirmation by the use of special methods of identification of individual chromosomes [8] in metaphases of the second maturation division, which were not used in this investigation.

The frequency of spontaneous nonseparation of chromosomes in oogenesis in albino rats from the Rappolovo nursery (0.8%) was thus considerably lower than the frequency of spontaneous aneuploidy in early embryos which, according to figures published by the writers elsewhere [2], is 8.4%. Chromosomal aberrations in embryos can be formed by nonseparation of chromosomes both in oogenesis and in spermatogenesis, as well as by mitotic nonseparation of chromosomes during fertilization and during the initial stages of cleavage [6, 10, 14]. These phenomena evidently play a more important role in spontaneous chromosomal embryopathies in rats of the "Rappolovo" colony. It must not be forgotten that spontaneous nonseparation of chromosomes in rat oogenesis is much less common than in mice [11], in agreement with the lower frequency of spontaneous pathology of embryogenesis in rats than in mice [1, 4, 14].

LITERATURE CITED

1. A. P. Dyban and I. M. Akimova, Arkh. Anat., No. 5, 36 (1967).
2. A. P. Dyban, L. D. Udalova, I. M. Akimova, et al., Arkh. Anat., No. 2, 22 (1971).

3. A. A. Prokof'eva-Bel'govskaya, Fundamentals of Human Cytogenetics [in Russian], Moscow (1969).
4. N. A. Chebotar', Farmakol. i Toksikol., No. 2, 221 (1967).
5. S. D. Biggers, D. G. Whittingham, and R. P. Donahue, Proc. Nat. Acad. Sci. (Washington), 58, 560 (1967).
6. R. P. Donahue, J. Exp. Zool., 180, 305 (1972).
7. H. Kalter, Genetics, 46, 874 (1961).
8. D. L. Odor, Am. J. Anat., 97, 461 (1955).
9. S. R. Patil, S. Merrick, and H. A. Lubs, Science, 173, 821 (1971).
10. G. Rohrborn, Humangenetik, 16, 123 (1972).
11. L. B. Russell, Progress in Medical Genetics, 2, 230 (1962).
12. A. G. Searle, C. E. Ford, and C. V. Beechey, Genet. Res., 18, 215 (1971).
13. A. K. Tarkowski, Cytogenetics, 5, 394 (1966).
14. A. D. Vickers, J. Reprod. Fertil., 20, 69 (1969).