FREQUENCY OF SPONTANEOUS CHROMOSOMAL ABERRATIONS IN THE EARLY POSTIMPLANTATION PERIOD OF EMBROYGENESIS IN MICE

V. S. Baranov and A. P. Dyban

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The frequency of spontaneous heteroploidy in C3HA mouse embryos on the 8th day of development is 2.7% (5 of 181 embryos), three embryos exhibited triploidy, and two had mosaicism of the chromosomes. In the karyotype of one mosaic there was an isochromosome. All the heteroploid embryos were anomalous and were in a state of resorption. The frequency of spontaneous chromosomal aberrations in embryogenesis is much lower in mice than in man. Considerable similarity was observed between spontaneous heteroploidy in mice and rats. A characteristic feature of mouse embryogenesis is the frequent formation of isochromosomes.

The study of spontaneous chromosomal aberrations in the embryogenesis of different mammals is of considerable interest from the points of view both of comparative cytogenetics and of developmental biology [3, 7].

In human embryogenesis chromosomal aberrations responsible for developmental anomalies and death of the embryos are frequently found. In spontaneous abortion in the early stages of pregnancy, for instance, the frequency of spontaneous heteroploidy may reach 30% [1, 6, 9]. In the earlier stages of development the frequency of spontaneous chromosomal aberrations in man is probably higher still [2, 5, 10].

Yet spontaneous heteroploidy is relatively less common during embryogenesis of laboratory rats [4]. The frequency of spontaneous heteroploidy in the embryogenesis of mice has not been adequately studied. By the end of the cleavage period heteroploidy was found in only 2.6% of mouse embryos [13]. The frequency of chromosomal aberrations during that period of mouse embryogenesis when such vital morphogenetic processes as gastrulation and neurulation take place and the axial complex is laid down has not been investigated.

The object of the present investigation was to study the karyotypes of mouse embryos in the early postimplantation period, i.e., on the 8th day of development, and to determine the frequency and character of spontaneous heteroploidy and its effect on embryogenesis.

EXPERIMENTAL METHOD

C3HA mice were used. Since the spontaneous embryonic mortality in laboratory mice undergoes considerable seasonal variations and depends on the conditions under which the animals are kept and on other external factors [8], all the observations were made at the same time of year and on the same colony of animals. The day of discovery of a vaginal plug was taken as the first day of pregnancy.

In the experiments of series I the fertility of the mouse colony was studied from the results obtained at the end of pregnancy. Females were autopsied on the 18th-19th day of pregnancy and the number of

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TABLE 1. Fertility of C3HA Mice

Day of pregnancy	Number of females investigated	Number of corpora lutea	Number of embryos dying before implantation		Number of implanted embryos			
			abs	%	total	%	dying abs.	dying %
8—9	22	217	27	12,4±2,2	190	87,5	9	4,7±1,5
18—19	13	142	25	17,6±3,1	117	82,3	17	14,5±3,2

Continuation

Number of implanted embryos living						Number of abnormal embryos dying before and after implantation	
total		normal		pathological		abs	%
abs	%	abs	%	abs	%		
181	95,3	156	86,1±2,5	25	13,8±2,5	61	28,1±3
100	85,4	100	100	—	—	42	29,5±3,8

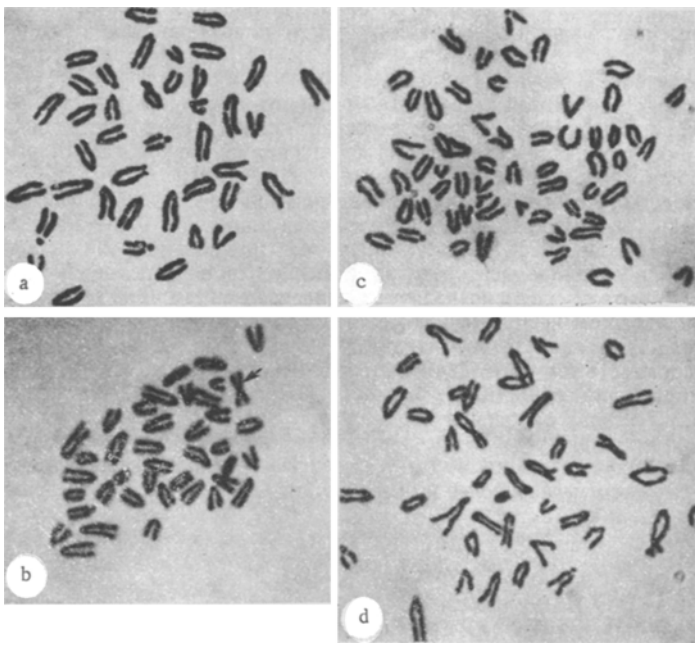

Fig. 1. Metaphase plates of pathological C3HA mouse embryos on 8th day of development: a) with normal modal chromosome number (2n=40); b) with isochromosome (shown by arrow); c) with triploidy (3n); d) with mosaicism of the chromosomes (2n= 40/41).

living and dead embryos and the number of corpora lutea in the ovaries were counted. The living fetuses were examined under the MBS-1 binocular loupe. Altogether 13 pregnant females were studied, and 117 fetuses were obtained from them.

Embryos at the 8th day of development were investigated in the experiments of series II. The uterine cornua were opened, the fetuses with the membranes were extracted under the control of the binocular loupe, and the fetuses were measured with a micrometer and photographed. Next, chromosome preparations were obtained from each embryo separately by the method of Wroblewska and Dyban [14]. In this

way it was possible to compare the microanatomical structure of each embryo with its karyotype. The chromosome preparations were stained with orcein lactate, studied, and photographed by means of the NF microscope with camera attachment (objective 100, oil immersion). On the average 15-20 metaphase plates were counted for each embryo. All metaphase plates were analyzed in fetuses with mosaicism of the chromosomes and in pathological fetuses. Altogether 181 fetuses were obtained from 22 pregnant females. These fetuses were studied microanatomically and cytogenetically.

EXPERIMENTAL RESULTS

On the 18th-19th day of pregnancy in C3HA mice 29.5% of the fetuses were dead (Table 1); 17.6% of the fetuses died before and during implantation, and the rest were eliminated at later stages of embryogenesis. All the living fetuses at this time of pregnancy appeared normal.

More detailed information on spontaneous death of the C3HA mouse embryos was obtained by analysis of the results on the 8th day of pregnancy, i.e., soon after implantation. As Table 1 shows, 12.4% of the ovulated oocytes were unfertilized or they died and were eliminated before implantation. In 9 cases implantation took place but the deciduomas were empty and some of them contained only the remnants of resorbed embryos which were unfit for cytogenetic analysis. Summing up for the early periods as a whole, i.e., before and during implantation, about 17% of the embryos died, in agreement with the results of the experiments of series I.

Implanted embryos (181) were alive, and 156 of them were normal and at the stage of neurulation and laying down of the axial complex of organs, while 25 (13.8%) showed obvious signs of pathology. They were approximately half the normal size, the neural grooves were greatly flattened, and as a rule the anlage of the allantois was absent. The presence of maternal blood in the cavity of the crypt and the absence of attachment of these germinal vesicles to the walls of the implantation chamber indicated their nonviability. All the normal embryos had the karyotype 2n=40. Among the malformed embryos 20 (80%) had the karyotype 2n=40, three (12%) had the karyotype 3n=60, and two (8%) had the karyotype 2n=40/41 (isochromosome, mosaic). Five embryos, or 2.7% of the total number and 20% of the number of malformed embryos, had chromosomal aberrations.

All the 156 female typically normal embryos at the 8th day of development thus had no visible numerical chromosomal aberrations. A normal karyotype was also characteristic of most (20 of 25) malformed embryos (Fig. 1a). Only 4 malformed embryos were heteroploid. Three embryos were triploid, and 2 had mosaicism of the chromosomes (Fig. 1b, c, d). In 1 mosaic approximately one-third of the metaphase plates had trisomy (2n=41), while the other had clones of cells with an unusual, small metacentric chromosome, although the number of chromosomes still remained 40, i.e., some cells of this embryo had trisomy on account of a superfluous small chromosome, consisting either of an isochromosome or centric fusion of two small autosomes.

The frequency of spontaneous heteroploidy in C3HA mice in the early postimplantation period (8th day) was only 2.7% of the total number of embryos investigated, i.e., it was within the same limits as in the rat embryos at the early postimplantation stages of development [4].

It can be concluded from these results that the frequency of spontaneous chromosomal aberrations during embryogenesis in mice, just as in rats, is much lower than in man, and that in these mouse-like rodents all embryos with numerical disturbances of the karyotype die during or soon after implantation.

The frequency of spontaneous triploidy in mice is known to depend on the genetic features of the strains and to vary between 0.46 and 7% [11, 12]. In C3HA mice the frequency of spontaneous triploidy was 1.6%. Predominance of triploids among embryos with chromosomal aberrations in the present experiments is in good agreement with results published in the literature [13] and it indicates the existence not only of quantitative, but also of considerable qualitative similarity between spontaneous heteropoloidy in mice and rats [4].

At the same time, spontaneous heteropoloidy in mice also had its own characteristic features and, in particular, a high frequency of formation of isochromosomes in the initial stages of cleavage. Chromosomal aberrations of this type are extremely rare in man [2] and are not found in rats [4]. The formation of isochromosomes in mice is a widespread phenomenon. Such cases have previously been observed in mice of lines RDF [13] and T_1IÉM (with centric fusion of autosomes).

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