SEX RATIO IN MOUSE EMBRYOS IN THE POSTIMPLANTATION PERIOD OF DEVELOPMENT

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The sex ratio of CBA mouse embryos was studied on the 10th and 18th days of development in order to discover any connection between the sex of the embryos and their mortality. The spontaneous embryonic mortality rate was found to increase until the 18th day of development; however, the rate was unconnected with the sex of the embryos and the distribution of embryos by sex at different stages of embryogenesis did not deviate from 1:1.

KEY WORDS: embryos; sex ratio; embryonic mortality.

There is an extensive literature on the study of the sex ratio in mammalian and human embryogenesis [1, 2, 6, 9, 10, 15] and, in particular, the connection between embryonic mortality and the sex of the embryo has been discussed. Contradictory opinions are expressed on this problem: Some workers consider that predominantly male or female embryos die during embryogenesis [11-13], whereas others have found no connection between mortality and sex of the embryo [16, 17].

Such widely different views are probably the result of the use of different methods to determine the sex of embryos. For instance, the sexing of embryos by determination of sex chromatin may distort the true sex ratio in the progeny [4, 5]. The sex of mammalian embryos can be established more accurately by investigating chromosomes in embryonic cells [14, 18].

This investigation was undertaken to determine the sex ratio in embryos at different stages of postimplantation development in order to study the connection between embryonic mortality and sex of the embryo.

EXPERIMENTAL METHOD

Male and female CBA mice were used. After mating the day of discovery of a vaginal plug was taken as the first day of pregnancy. The females were killed on the 10th and 18th days of pregnancy.

Chromosome preparations from cells of 10-day embryos were obtained by the method of Wroblewska and Dyban [18] and then trypsinized (0.01% solution of trypsin in phosphate buffer, pH 6.8, at 37°C) and stained with standard Giemsa solution in phosphate buffer, pH 6.8.

TABLE 1. Distribution of 10-Day and 18-Day Mouse Embryos by Sex

Age of embryos (in days)	Number of embryos	Sex ratio	x^2	P
10	221♂:256♀	86,3♂:100♀	2,48	>0,1
18	303♂:319♀	94,9♂:100♀	0,4	>0,2

Sex chromosomes were identified in accordance with the recommendations of the Committee on Standardized Genetic Nomenclature for Mice [8].

Embryos at the 18th day of development were removed from the uterus and, after laparotomy, their gonads were investigated with the MBS-1 microscope.

Altogether 210 pregnant mice were used: Of these 116 were killed on the 10th day and 94 on the 18th day of pregnancy. The total number of 10-day embroys examined cytogenetically

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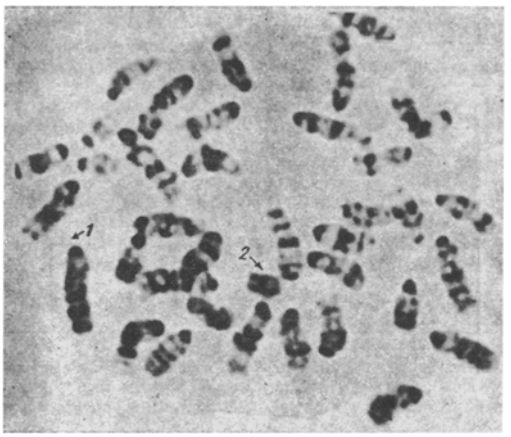

Fig. 1. Chromosome set of 10-day mouse embryo. Sex chromosomes indicated by arrows: 1) X chromosome; 2) Y chromosome.

TABLE 2. Postimplantation Mortality of 10-Day and 18-Day Mouse Embryos

Number of pregnancies	Age of embryos (in days)	Number of implanted embryos	Number of embryos dying after implantation	
			absolute	% ±
116	10	846	29	3,42±0,62
94	18	674	58	8,60±1,07

was 477. The sex of 622 18-day embryos was determined macroscopically.

EXPERIMENTAL RESULTS

The 10-day embryos were sexed by the study of their sex chromosomes which, after staining by the trypsin–Giemsa method, can be identified sufficiently easily (Fig. 1).

The Y chromosome, for instance, stains more intensively than the other chromosomes of the set and it has no pericentromeric heterochromatin. X chromosomes are identified by the characteristic arrangement of their bands [8].

As Table 1 shows, the sex ratio of the embryos (the number of males to 100 females) did not significantly differ from the 1:1 distribution ($x^2 = 2.48$, $P > 0.1$). The same sex ratio also was observed later during development on the 18th day ($x^2 = 0.4$, $P > 0.2$).

Investigation of postimplantation deaths of embryos at these same times of development showed that the embryonic mortality on the 10th day was 3.4% and on the 18th day it had increased to 8.6% (Table 2).

The results of this investigation thus showed that the sex ratio in mouse embryos at different stages of postimplantation development does not diverge from the 1:1 distribution. The increase in embryonic mortality toward the end of this period of development does not disturb this distribution of embryos by sex.

These findings are in good agreement with the results of investigations of the sex distribution of mouse embryos of the same ages but different strains undertaken by Vickers [16, 17]. In early mouse embryos, i.e., in the preimplantation period of development, the sex ratio likewise does not diverge from the 1:1 distribution [10].

The study of human embryos during the first 3 months of development and of children during the first years of life has also shown no divergence of their sex ratio from the 1:1 distribution [1, 3, 5, 7].

It can thus be concluded from the analysis of the experimental results and data in the literature that spontaneous embryonic mortality is unconnected with sex of the embryos and that the sex distribution of embryos at different stages of development does not diverge from the 1:1 ratio.

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