SEX RATIO IN HUMAN EMBRYOS FROM THERAPEUTIC ABORTION STATISTICS

A. A. Kostrova

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The sex ratio was studied in human embryos by identification of the sex chromatin and by karyotype analysis in cultures of embryonic fibroblasts. Investigation of 2000 embryos obtained by medical abortion showed a sex ratio of 104.9 ♂: 100♀, which corresponds to a ratio of 1:1 and to the secondary sex ratio with a 5% level of significance. The rate of incidence of cells with sex chromatin in male embryos was 0 and in female it varied from 14 to 52%. The distribution of sex chromatin in the cells of female embryos is represented by a curve with positive asymmetry.

The view was held until recently that male embryos die in large numbers from sex-linked lethal mutations. The existence of a preponderance of male embryos has been reported in several investigations conducted by different methods on material obtained after spontaneous and therapeutic abortion [4, 6, 7]. However, in a recent determination of the sex ratio by a more refined technique, the author [1] and also Kukharenko [5], have shown that the ratio does not differ from 1:1. The limits of variations of the sex ratio among embryos calculated in a previous paper [4] were 100-108 boys to 100 girls.

The object of this investigation was to verify these calculations experimentally and to determine the sex ratio in man in the embryonic period more exactly.

EXPERIMENTAL

The sex ratio was determined by detection of sex chromatin and by karyotype analysis. Embryonic tissues were cultivated for this purpose.

TABLE 1. Results of Analysis of Sex Ratio in Human Embryos

No. of batch of 100	No. of embryos		No. of batch of 100	No. of embryos	
	male	female		male	female
1	46	54	11	56	44
2	54	46	12	42	58
3	41	59	13	42	58
4	50	50	14	51	49
5	53	47	15	51	49
6	54	46	16	43	57
7	54	46	17	52	48
8	54	46	18	57	43
9	53	47	19	60	40
10	57	43	20	54	46

Pieces of skin muscles with a total volume of about 2 cm^3, obtained after therapeutic abortion from healthy women between the 6th and 12th weeks of pregnancy, were taken from the maternity homes to the laboratory in penicillin flasks containing Hanks's solution. From 4-6 h after the material was obtained the embryonic tissue was cut up in the same flasks with scissors to a size of 1-2 mm^3. The minced mass was covered with a mixture of trypsin and versene (3:1) equal to five times its volume and incubated for 15-20 min. The mixture was then removed and 6-8 ml of a culture fluid consisting of medium No. 199 (80%), bovine serum (20%), and penicillin (300 units per ml medium) was added to each flask. The resulting suspension from each embryo was poured into two penicillin flasks with coverslips in the bottom.

The cells were cultivated at 37°C for 48-50 h. If the specimens were to be prepared for examination for sex chromatin, the nutrient medium was poured off and 3-4 ml of a mixture of ethanol with acetic acid (3:1) was added to each flask.

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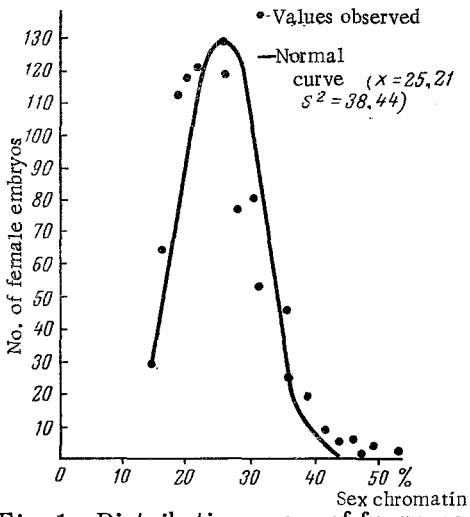

Fig. 1. Distribution curve of frequency of cells with sex chromatin among female embryos. Abscissa – percent of cells containing sex chromatin; ordinate – number of female embryos; continuous line – normal curve; dots denote values actually observed (arithmetic mean x = 25.21, dispersion S^2 = 38.44).

In the case of karyotype analysis, 4 h before fixation 0.2 ml of colchincine solution (final concentration 10^{-6}) was added. The nutrient medium was withdrawn from the flasks after 48-50 h and 5 ml of a 0.55% solution of the potassium chloride was added for 15-18 min. After removal of the solution the coverslips were fixed in the same way as for the detection of sex chromatin.

The specimens were stained with 2% orcein acetate for 10 min, dehydrated in alcohols, cleared in xylol, and mounted in balsam.

Sex chromatin was determined in oval nuclei of average size with a finely granular structure. An intensely stained oval particle lying at the edge of the nuclear membrane was taken as sex chromatin. To estimate the incidence of cells with sex chromatin, 50-100 cells were studied from each embryo.

Metaphases satisfying all the requirements for this purpose were chosen for karyotype analysis [3]. At least five cells from each embryo were analysed, so that the sex could be reliably determined from the chromosomes.

EXPERIMENTAL RESULTS

Altogether 2000 embryos were studied for sex chromatin. The incidence of cells with sex chromatin in some embryos was 0, while in the others it varied from 14 to 52%. Absence of sex chromatin characteristic of male individuals can thus be clearly demonstrated after cultivation of embryonic tissues. Because of the high percentage of cells with sex chromatin in the female embryos, the sex ratio can be studied satisfactorily by this method.

The distribution of female embryos by the percentage of embryonic fibroblasts in the culture containing sex chromatin is shown in Fig. 1.

The resulting curve was an almost normal distribution but showed positive asymmetry. Its characteristics were as follows: $\bar{x}$ = 25.21; S = 6.20; S^2 = 38.44; A = 0.666 ± 0.231; E = 0.306 ± 0.775. The presence of asymmetry suggests a significant gap between the percentage of sex chromatin in the male and female embryos. The absence of an excess in the distribution of the sex chromatin level among the female embryos evidently means that the distribution was not random and was independent of various influences.

The validity of sex determination in embryos by counting sex chromatin in cultures of fibroblasts was also confirmed by sex determination from chromosomes in 268 embryos. In every case the results of sex determination by detection of sex chromatin agreed with those obtained by karyotype analysis.

The results obtained for the sex ratio in chronological order for each 100 embryos are given in Table 1 in order to show the range of variation with small samples.

The ratio between male and female embryos in absolute figures was 1024♂:976♀ or 104.9♂:100♀, respectively. The results do not differ significantly from the ratio 1:1 or from the secondary sex ratio for Moscow [2] with a 5% level of significance. They also lie within the range of variation of the sex ratio determined previously in embryos by other methods [1].

It can accordingly be concluded from this investigation that the sex ratio in human embryos is close or equal to the secondary sex ratio of 105-106 boys to 100 girls.

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