BRIEF COMMUNICATION

AMNIOTIC SEX CHROMATIN AND FOETAL SEXING IN THE MOUSE

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Summary. Sex chromatin is present in 67 to 90% of the ectodermal cell nuclei in the female mouse amnion. A similar body is found in 2 to 15% of these nuclei in the male. This criterion is a reliable indicator of the sex of the foetus from the 11th day of gestation onwards.

Sex chromatin is found in many of the cell nuclei of females, but is absent from the nuclei of males. In a suitable tissue, the presence or absence of nuclear sex chromatin is a reliable indicator of the genetic sex of the individual. The nuclei of mouse cells usually contain so much heterochromatin that sex chromatin cannot be detected (Moore, 1966); nevertheless, sex chromatin should be present in suitable tissues, because the behaviour of the X chromosomes in the mouse is similar to that of other species. One X chromosome in each cell of the female mouse is genetically inactive (Lyon 1961), replicates its DNA late in the S-phase of the cell cycle (Galton & Holt, 1965), and is heterochromatic in the prophase stage of mitosis (Ohno & Hauschka, 1960). These features are associated with the appearance of sex chromatin in other species and, therefore, provide indirect evidence for sex chromatin formation in the mouse. Kenney & Mittwoch (1965) found sex chromatin in cultured fibroblasts from female mice, but only in 10% of 'clear' cells with few chromocentres; these 'clear' cells constituted about half of the total cell population. Bianchi & de Bianchi (1966) and Farias, Kajii & Gardner (1967) sexed rat foetuses by observation of sex chromatin in the amnion; Bianchi & de Bianchi (1966) state, although without further details, that the method can be used in the mouse. The amnion is known to be one of the most favourable tissues for nuclear chromatin analysis: the human female amnion contains 80 to 96% sex chromatin positive nuclei (Klinger, 1966). It therefore seemed worthwhile to examine the mouse amnion to discover firstly whether sex chromatin was visible, and secondly whether it could be used for sexing foetuses.

Pregnant mice were killed 10½, 11½, 13½ and 17½ days after ovulation. The amnion was dissected out, transferred in a pipette to a clean, albumen-coated slide, and flattened with a paintbrush and fine forceps; care was needed in handling the amnion from early foetuses. The amnion was fixed in 95% alcohol and coated with celloidin (Geneva Conference, 1966), stained for 15 min in 2% lactic-acetic-orcein, dehydrated and mounted in Canada balsam.

The sex of the 10½-, 11½- and 13½-day-old foetuses was established by chromosome analysis of at least ten metaphase plates, obtained from the whole foetus or the foetal liver by the method of Ford & Woollam (1963). The female karyotype contains two small chromosomes whilst the male karyotype contains three small chromosomes: the Y plus the two smallest autosomes (Stich & Hsu, 1960). The preparations of chromosomes and amniotic membranes were coded and studied 'blind'. The sex of the 17½-day-old foetuses was established by inspection of the reproductive system.

TABLE 1
THE PERCENTAGE OF ECTODERMAL CELL
NUCLEI WITH SEX CHROMATIN IN THE
MOUSE AMNION

Age of foetus (days)	% of nuclei with sex chromatin	
	Female	Male
10½	81.3 ± 1.4*	3.7 ± 0.6
11½	86.8 ± 0.6	6.8 ± 0.6
13½	77.5 ± 0.8	11.3 ± 1.2
17½	73.5 ± 1.7	8.2 ± 1.0

* S.E. of the mean.

TABLE 2
THE NUMBER OF MOUSE FOETUSES SEXED BY NUCLEAR
CHROMATIN AND ALSO BY KARYOTYPE OR MORPHOLOGY

Age of foetus (days)	Nuclear chromatin		Karyotype		Morphology	
	Female	Male	Female	Male	Female	Male
10½	6	9	6	9		
11½	7	13	6*	11*		
13½	12	7	12	6*		
17½	14	17			14	17
10½-17½	39	46	24	26	14	17

* Some chromosome cultures failed.

Observations of the amnion were made with bright-field and phase-contrast microscopy; the former gave more satisfactory results with the 10½- and 11½-day-old amnion. The mouse amnion was found to consist of a continuous sheet of flattened ectodermal cells and an equivalent number of scattered mesodermal cells. The mesodermal cell nuclei contained many large chromocentres and no clear sexual dimorphism could be demonstrated. The ectodermal cell nuclei contained only a few small particles of chromatin and also sometimes contained a chromocentre, approximately $1.25 \times 0.9 \mu$ in size, which was morphologically identical with sex chromatin. The amniotic membranes were classified into those with many sex chromatin bodies and those with few sex chromatin bodies; the former were assumed to come from female foetuses and the latter from male foetuses (Plate 1). Detailed counts of

PLATE 1

FIG. 1. Amnion of a female mouse foetus, $13\frac{1}{2}$ days of gestation ($\times 240$).

FIG. 2. Amnion of a male mouse foetus, $13\frac{1}{2}$ days of gestation ($\times 240$).

(Facing p. 504)

sex chromatin were made on a sample of six foetuses of either sex from each age group. One hundred ectodermal cell nuclei from each individual were scored for the presence or absence of a sex chromatin body adjacent to the nuclear membrane and the results are shown in Table 1. Sex chromatin was present in 67 to 90% of the nuclei of females, whilst a similar body was present in only 2 to 15% of the nuclei of males.

Eighty-five foetuses were sexed in the experiment, and in every case the sex, as determined by the proportion of cells with sex chromatin in the amnion, was correct as judged by karyotype or morphology (Table 2). There were no errors or doubtful classifications; every foetus handled could be sexed on the basis of the amniotic chromatin, but the chromosome cultures failed in four cases. These results show that the amnion provides excellent material for the demonstration of sex chromatin in the mouse, and can be used for sexing foetuses from the 11th day of gestation onwards. The amnion from younger foetuses is too small to handle reliably, but it does show sexual dimorphism of nuclear chromatin (unpublished observations).

The existing methods of sexing mouse foetuses are unsatisfactory: the gonads are not clearly differentiated until the 15th day of gestation and although chromosome analysis can be applied at any age, it is time-consuming and involves destruction of the foetus. The presence of sex chromatin in the mouse amnion provides an alternative method of sexing foetuses which is rapid and reliable and leaves the foetus intact for any other procedures.

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