

DELAYED FERTILIZATION AND CHROMOSOMAL ANOMALIES IN MOUSE EMBRYOS

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Summary. Chromosomes were studied in mouse embryos and foetuses recovered after superovulation. The spontaneous incidence of chromosomal anomalies was 2.6%; a delay of 7 to 13 hr before fertilization produced 3.9% anomalies. The most striking feature was a nine-fold increase in the incidence of triploidy after delayed fertilization. The chromosomal anomalies included isochromosomes and minute chromosomes. Some anomalous individuals survive implantation and the fate of such foetuses is discussed.

INTRODUCTION

Three main events lead to chromosomal anomalies at the time of fertilization: meiotic errors, fertilization errors and cleavage errors. In general terms: meiotic errors give rise to karyotypes with numerical or structural changes, fertilization errors produce karyotypes with numerical changes, and cleavage errors produce mosaic embryos (although complete suppression of the first cleavage division produces a tetraploid embryo).

Fertilization of ageing eggs (delayed fertilization) leads to an increased incidence of chromosomal anomalies in amphibians, rats, rabbits and pigs. Witschi & Laguens (1963) delayed fertilization of frog and toad eggs for 3 to 5 days and found that embryos recovered after hatching showed an increased incidence of meiotic, fertilization and cleavage errors. Austin & Braden (1953) found that delaying fertilization for some hours after ovulation increased the incidence of polyspermy from 1.5% to 9.2% in rats, and from 0.5% to 13% in rabbits. Shaver & Carr (1967) made similar observations on rabbits. Austin (1967), in a later study on rabbits, found that delayed fertilization resulted in a three-fold increase in the incidence of hypodiploid embryos. These anomalies were produced by disintegration of the second meiotic spindle in ageing eggs. After delayed fertilization in the sow, Thibault (1959) found 20% digyny plus 11% diandry, Hancock (1959) found 40% triploid embryos, and Bomsel-Helmreich (1965) found 6% triploid embryos.

However, Gates & Beatty (1954) and Braden & Austin (1954) found that delayed fertilization in the mouse did not increase the incidence of triploidy. Since an improved method for preparing chromosomes from mouse embryos has been developed recently (Tarkowski, 1966), it seemed worthwhile to re-investigate the effect of delayed fertilization on mouse embryos in spite of these negative reports. The fate of chromosomally abnormal individuals is of interest, and so post-implantation material was also studied.

MATERIAL AND METHODS

These results are derived from the same material which provided the sex-ratio data reported in a separate communication (Vickers, 1969). PDE mice, an albino strain, were induced to superovulate; the control group was allowed to mate at the time of ovulation but the delayed fertilization groups were not allowed to mate except between 5 and 11 hr after ovulation.

Embryos were recovered 3 to 4 days after ovulation and chromosome preparations were made as previously described (Vickers, 1967a). Most chromosome counts were precise, but some were estimated as diploid ± 2 chromosomes.

A few chromosome preparations were made $9\frac{1}{2}$ to $11\frac{1}{2}$ days after ovulation by a squash technique (Piko & Bomsel-Helmreich, 1960) but the results were unsatisfactory and so most of the preparations at this time were made from the whole foetus or foetal liver by the method of Ford & Woollam (1963). The technique was modified for the younger foetuses as follows: the foetus was left intact until after fixation and then broken up by gentle pipetting in 45% acetic acid before air-drying. The modification was always successful with $9\frac{1}{2}$ - and $10\frac{1}{2}$ -day-old foetuses and could even be applied to smaller quantities of tissue, for example the tail of a $9\frac{1}{2}$ -day-old foetus. The modification was, however, unsuitable for use on $11\frac{1}{2}$ -day-old foetuses because they did not break up readily after fixation. Amniotic preparations were made from most of the foetuses recovered and the sex chromatin patterns were studied as previously described (Vickers, 1967b).

Two microscope slides were prepared from each foetus and, if possible, at least ten well-spread metaphases were studied from each slide. Metaphases were selected at a magnification of $\times 100$ and studied in detail at a magnification of $\times 900$. Karyotyping was not attempted. The foetus was recorded as diploid when most of the metaphases contained 40 chromosomes. Occasional cells with 39 or fewer chromosomes were presumed to be artefacts. Where fewer than twenty metaphases were suitable for analysis, the foetus was recorded as '? diploid' because of the possibility of undetected mosaicism.

RESULTS

Control group $3\frac{1}{2}$ days after ovulation

The results of the chromosomal analysis of 309 embryos obtained from thirty-two pregnant mice are presented in Tables 1 and 2. These results include those presented previously (Vickers, 1967a).

The single haploid blastocyst appeared degenerate on recovery and six of the thirty-two cells had pycnotic nuclei.

There were two trisomic embryos; one of them had a double blastocoele, and it is tempting to speculate on the association of these two events in one embryo. In the present series of investigations, trisomy occurred in three out of 1000 embryos and a double blastocoele in two out of 309 embryos so that the probability of finding these two independent events in the same embryo is 0.000019. The association was not invariable: one of the embryos with a double blastocoele was diploid, while the other trisomic embryo formed a normal blastocyst.

TABLE 1
THE CHROMOSOME COMPLEMENTS OF EIGHT SPONTANEOUS HETEROPOID EMBRYOS RECOVERED 3½ DAYS AFTER OVULATION

Anomaly	Total cells in embryo	No. of cells assigned to chromosome class										Sex	
		20	34	37§	No. of chromosomes				41	60	60±2		
1n†	32	3											?
3n	54												?
2n+1	55									1		3	XY
2n+1	84									1			XY
2n*‡	56							3					XY
Mosaic	37		1	1	1								?
2n†	34									1			XY
2n/2n†	58						6	2					XX

* Complement includes a minute chromosome.
 † Complement includes an isochromosome.
 ‡ Siblings.
 § Complement includes two minute chromosomes.

TABLE 2
THE DISTRIBUTION OF CONTROL MOUSE EMBRYOS 3½ DAYS AFTER OVULATION

Type	No. embryos	% embryos typed
Presumed normal:	301	97.41
2n	226	73.13
2n±2	75	24.28
Heteroploid:	8	2.59
1n	1	0.32
3n	1	0.32
2n+1	2	0.65
2n*	1	0.32
Mosaic	2	0.65
2n†	1	0.32

* Complement includes a minute chromosome.
 † Complement includes an isochromosome.

Minute chromosomes were found in two embryos (Pl. 1, Figs. 1 and 2); one of these embryos was a complex hypodiploid mosaic. The minute chromosomes may have been produced by a deletion or a translocation.

An isochromosome was present in two embryos (Pl. 1, Figs. 3 and 4); one of these embryos was a mosaic and it can be deduced that the isochromosome arose at the second or third cleavage division (probably from a misdivision of the centromere).

Most of the heteroploid embryos were of normal morphological appearance both before and after fixation and might well have continued to develop; the exceptions were the haploid embryo and one of the trisomic embryos.

Delayed group 3 to 4 days after ovulation

Twenty-nine pregnant mice provided 207 embryos suitable for chromosomal analysis (Tables 3 and 4).

TABLE 3

THE CHROMOSOME COMPLEMENTS OF EIGHT HETEROPLOID EMBRYOS RECOVERED 3 TO 4 DAYS AFTER DELAYED FERTILIZATION

Anomaly	Total cells in embryo	No. of cells assigned to chromosome class					Sex
		No. of chromosomes					
		39	50 to 60	60	60±2	80	
3n	35				1		?
3n	58				7		XXX
3n	18		2	1	2		?
3n	18		1	2			?
3n*	59			1			XXX
3n	93			2	1		?
4n	27					1	?
2n-1*	55	3					XX or XO

* Siblings.

TABLE 4

THE DISTRIBUTION OF MOUSE EMBRYOS RECOVERED 3 TO 4 DAYS AFTER DELAYED FERTILIZATION

Type	No. embryos	% embryos typed
Presumed normal:	199	96.14
2n	120	57.97
2n±2	79	38.16
Heteroploid:	8	3.86
3n	6	2.90
4n	1	0.48
2n-1	1	0.48

TABLE 5

THE CHROMOSOME COMPLEMENTS OF FIVE HETEROPLOID FOETUSES RECOVERED 9½ TO 11½ DAYS AFTER DELAYED FERTILIZATION

Anomaly	No. of cells assigned to chromosome class						Sex
	No. of chromosomes						
	39	40	41	40*	50 to 60	60	
3n					4	5	XXX
2n-1	16						XO
2n/2n (min)*†		48		2			XY
2n/2n+1/2n-1†	6	39	4				XY
2n/2n+1/2n-1	6	46	3				XX

* Complement includes a minute chromosome.

† Siblings.

The six triploid embryos and the one monosomic embryo all appeared morphologically normal before and after fixation. One tetraploid embryo, which appeared to be normal before fixation, proved to have some abnormally large nuclei and some pycnotic nuclei and was probably degenerating.

PLATE 1

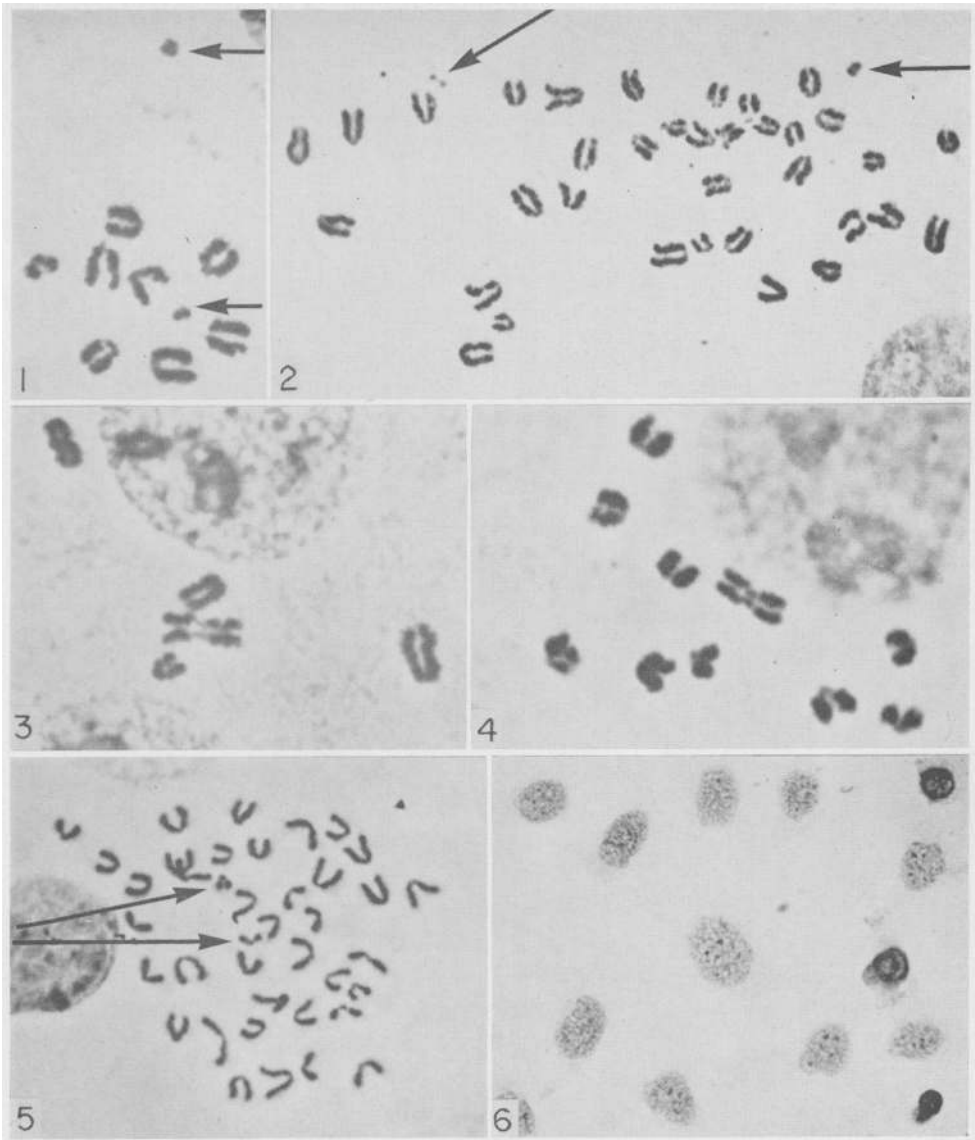


FIG. 1. Minute chromosomes (arrowed) in two adjacent metaphases from a control embryo: $2n(\text{min})$. $\times 2120$.

FIG. 2. Two minute chromosomes (arrowed) in one metaphase from a control embryo: mosaic. $\times 1350$.

FIG. 3. Isochromosome in a metaphase from a control embryo. $\times 2120$.

FIG. 4. Isochromosome in a different metaphase from the same embryo. $\times 2120$.

FIG. 5. XO foetus recovered $10\frac{1}{2}$ days after delayed fertilization: metaphase containing 39 chromosomes with two small autosomes (arrowed) but no Y chromosome. $\times 1350$.

FIG. 6. No sex chromatin in the amnion from the XO foetus. $\times 540$.

(Facing p. 72)

TABLE 6
THE DISTRIBUTION OF MOUSE FOETUSES RECOVERED
9½ TO 11½ DAYS AFTER DELAYED FERTILIZATION

Type	No. foetuses	% foetuses typed
Presumed normal:	90	94.74
2n	60	63.17
2n (?)	30	31.58
Heteroploid:	5	5.26
3n	1	1.05
2n-1	1	1.05
Mosaic	3	3.16

Delayed group 9½ to 11½ days after ovulation

This age group was chosen with a view to determining the fate of triploid embryos. No control group was studied since the results 3½ days after ovulation had shown that triploidy was very rare in control animals. Eleven pregnant mice provided ninety-five foetuses suitable for chromosomal analysis (Tables 5 and 6).

The triploid foetus was structurally normal but smaller and paler than its ten diploid siblings, no heart beat could be detected and it seems likely that this foetus was dying. The sex chromatin constitution was not identified.

No siblings were available for developmental comparison with the single monosomic foetus. Each metaphase studied from this foetus showed 39 chromosomes including two very small autosomes but no Y chromosome (Pl. 1, Fig. 5). Independent examination of the amnion showed a male pattern of nuclear chromatin. Eight per cent of ectodermal cells contained sex chromatin-like bodies (Pl. 1, Fig. 6). These findings suggest that the foetus had an XO sex chromosome constitution.

There were three mosaic foetuses, all of which appeared to be developing normally. Two of the mosaic foetuses probably arose by unequal separation of the chromosomes at one of the early cleavage divisions. The minute chromosome in the other mosaic foetus was probably the result of a deletion or a translocation in one cell during development.

TABLE 7
THE EFFECT OF DELAYED FERTILIZATION AND IMPLANTATION ON
VARIOUS TYPES OF CHROMOSOMAL ANOMALY

Origin of anomaly	<i>Anomalous young, expressed as % young typed</i>		
	<i>3½ day Control</i>	<i>3 to 4 day Delay</i>	<i>9½ to 11½ day Delay</i>
Fertilization	0.65	2.90	1.05
Meiosis	1.28	0.48	1.05
Cleavage	0.32	0.48	3.16
Meiosis + cleavage	0.32		
Total	2.57	3.86	5.26

The chromosomal anomalies found were classified according to the type of origin (Table 7); where there are theoretical alternatives, the most likely origin has been given. The XO foetus has been classed as a meiotic error although Russell (1961) suggests that XO mice arise by loss of the paternal sex chromosome from the pronucleus.

DISCUSSION

The abnormalities which can be detected are limited because all the chromosomes of the mouse are telocentric or acrocentric and very few can be recognized with certainty. Structural changes in individual chromosomes are unlikely to be detected unless the product is larger or smaller than all the other chromosomes present, but numerical changes in the karyotype will be detected without difficulty.

Most of the chromosomal anomalies reported here are paralleled in work on other species. Boué & Boué (1966) found human spontaneous abortuses with mosaics of $2n/2n+1/2n-1$ and of $2n/2n+1$ + minute. There are, however, no previous reports of an isochromosome occurring in an embryo. Isochromosomes of the sex chromosomes have been found in adult man (Moore, 1966; Jacobs & Ross, 1966), but there are no previous reports of isochromosomes in any other mammalian species. It is not clear whether the PDE mouse embryos with an isochromosome would have survived.

Delayed fertilization can be seen to increase the incidence of fertilization errors. The nine-fold increase in the incidence of triploidy is statistically significant ($\chi^2 = 6.14$; $P < 0.02$). This shows that ageing of the PDE mouse egg affects either the block to polyspermy or the formation of the second polar body. The latter appears more likely because of the sex chromosome constitution of two of the triploid embryos (XXX), and also because Braden & Austin (1954) found that mouse triploids, whether spontaneous or resulting from heat shock, were all the result of digyny. Delayed fertilization increases the incidence of triploidy as a result of diandry in the rat and rabbit (Austin & Braden, 1953) and as a result of digyny and diandry in the pig (Thibault, 1959). As already stated, Gates & Beatty (1954) and Braden & Austin (1954) found no significant increase in the incidence of triploidy in mouse embryos following delayed fertilization. The discrepancy between their results and the present findings may have arisen because they used immature mice or because of differences in the strain of mouse used. The incidence of spontaneous triploidy is affected by the strain in both mice (Beatty, 1957) and rats (Piko, 1958); also the incidence of triploidy after delayed fertilization is affected by the strain in rats (Piko, 1958).

Delayed fertilization in other species has a much more striking effect on the incidence of chromosomal anomalies. The differences in response probably reflect differences in the mechanics of fertilization between various species and between various strains.

The proportion of cleavage errors appears from Table 7 to be increased in the older age group but this finding needs further confirmation. The table also shows that some chromosomally abnormal individuals survive implanta-

tion. The mosaic foetuses might have continued to develop normally because of the high proportion of diploid cells present. The monosomic foetus would almost certainly have survived since XO is the commonest chromosomal anomaly in the mouse, though other types of monosomy are very rare. The 10½-day-old triploid found in these experiments was clearly abortive; Fishberg & Beatty (1951) found seven (+one?) triploid mouse foetuses which were normal in appearance but smaller than their diploid siblings after 9½ days of gestation. Edwards (1958) found no aneuploid young born after a colchicine treatment which induced 11% of triploid embryos. Triploid foetuses are almost invariably lost during the first half of gestation in rats (Piko & Bomsel-Helmreich, 1960), pigs and rabbits (Bomsel-Helmreich, 1965) and man (Edwards, Yuncken, Rushton, Richards & Mittwoch, 1967). It appears that the same is true of mice.

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