27. Cytogenetic Evidence of the Giant Diploid Oocytes Capable of Developing into Digynic Triploids in the Chinese Hamster

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Various embryological findings clearly indicate that the giant eggs twice the normal size are capable of developing into giant embryos in wide varieties of animals (Wilson, 1928; Austin, 1961). The giant ova may contain two nuclei or only one double the normal size. Some of these studies with the species of lower classes have shown that they grow as triploids with double female genomes, i.e., digynic triploids (Wilson, 1928; Uzzell et al., 1975). However, their development as triploids has not been chromosomally confirmed as yet in mammals.

Recently, the parental origins and the causal mechanisms of human triploidy have been analyzed by the use of heteromorphic marker chromosomes. As to the digynic triploids, the predominance of these caused by the suppression of the first polar body emission is strongly emphasized rather ignoring the giant diploid ova (Jacobs et al., 1978). However, the importance of the giant oocytes as a cause of human triploidy should not be overlooked, since binucleated oocytes have been seen in human ovaries at a considerable frequency (Hartman, 1926; Pankratz, 1938; Bacsich, 1949; Kennedy and Donahue, 1969).

We have been able to collect a number of giant oocytes and zygotes in the Chinese hamsters used in our several experimental projects. This paper presents their cytogenetical aspects during pre- and postzygotic stages with discussion on their biological significance in relation to the digynic triploidy in man.

Materials and methods. The eggs at four different stages, i.e., primary and secondary oocytes and one-cell and two-cell zygotes, were collected from sexually mature Chinese hamsters (5–8 months old) raised in our closed colony under an optimum condition: Room temperature at 23±2°C, humidity within 50–60%, and 14 hrs illumination from 5:00 to 19:00. Under these conditions, the females maintained constant 4-day cycles. The surge of LH usually occurs at 14:00–15:00 of the day of proestrus and ovulation does at 3:30–4:30 the following morning. Therefore, it was possible to determine
the right time of the intraperitoneal injection of colchicine and of the dissection for collecting eggs.

Primary oocytes were obtained from mature follicles immediately after the germinal vesicle breakdown, at 17:00 of the day of proestrus. The oocytes were cultured in a medium with Eagle's MEM containing 30% fetal calf serum and 0.025 μg/ml colcemid for about 3 hrs at 37°C. The majority of secondary oocytes were squeezed out from the ampullar region of oviducts about 5 hrs after ovulation on the day of estrus, and some were collected by pricking the mature follicles which missed normal ovulation by the time of the dissection. One-cell and two-cell zygotes were collected from oviducts of copulated females by flushing about 26 hrs and 59 hrs after ovulation, respectively. Three hours for the one-cell zygotes and five hours for the two-cell zygotes prior to the dissection, these females had been injected intraperitoneally with 5 μg/g b.w. colchicine in order to arrest cleavage divisions.

Chromosome preparations were made by the method described previously by us (Kamiguchi et al., 1976, 1978). Using this method, more than 90% of both the oocytes and zygotes were karyotyped successfully.

Results. A total of 37 giant eggs were found in 10,321 eggs obtained from 1,146 females. These included 6 primary and 18 secondary oocytes and 7 one-cell and 6 two-cell zygotes. They were about twice the normal size. Their development looked normal as compared with their sibs (Fig. 1).

A total of 31 giant eggs were karyotyped, as shown in Table I. Two giant primary oocytes at metaphase had 22 bivalents (tetrads) instead of 11 in a normal oocyte (Fig. 2). Their chiasma configuration appeared normal. Among 18 secondary oocytes, 17 were observed to have 22 dyads in a single metaphase plate (Fig. 3), but the re-
Table I. Chromosomal aspects of giant eggs in various stages in the Chinese hamster

<table>
<thead>
<tr>
<th></th>
<th>No. of giant eggs</th>
<th>Chromosomal aspects</th>
<th>No. of cases</th>
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</thead>
<tbody>
<tr>
<td>Primary oocyte</td>
<td>2</td>
<td>22 tetrads</td>
<td>2</td>
</tr>
<tr>
<td>Secondary oocyte</td>
<td>18</td>
<td>22 dyads</td>
<td>18</td>
</tr>
<tr>
<td>One-cell zygote</td>
<td>6</td>
<td>33,XXX</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33,XXY</td>
<td>2</td>
</tr>
<tr>
<td>Two-cell zygote</td>
<td>5</td>
<td>33,XXX</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33,XXY</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 2. A metaphase plate of meiosis I of a giant oocyte showing 22 bivalents (tetrads) which indicates single spindle structure. The karyotype shows duplicated bivalent chromosome complements and their normal chiasma configuration.

Fig. 3. A metaphase plate of meiosis II of a giant oocyte showing 22 dyads which indicates single spindle structure. The karyotype shows duplicated dyad chromosome complements and their normal features.
maining case displayed two haploid plates distinctly separated from each other, indicating that the binuclear state had been maintained. It was confirmed that all giant one-cell zygotes were triploids. In these slides, a single plate with double female genomes and one with a single male genome could be analyzed separately (Fig. 4), owing to that the first somatic division was arrested by colchicine before the syngamy took place. In such samples, the condensation of paternal chromosomes were consistently delayed, and thus parental origin of the chromosomal groups could be pointed out by their length. Five giant two-cell zygotes exhibited that each blastomere had a complete triploid set of chromosomes. It was also confirmed that they bore the tail of a fertilized spermatozoon (Fig. 5). The frequency distribution of the two possible sex variants in eleven developing giant triploids was 5 cases of XXX and 6 cases of XXY, giving a ratio nearly 1:1, as expected (Table I).

Discussion. The present paper offers the first clear evidence on the chromosomal aspect of the giant eggs during maturation and early zygotic stages. Their embryological and cytogenetical features defi-
nitely show that they are capable of normal development at least up to the 2nd cleavage metaphase. Austin and Amoroso (1959) have also observed a giant egg developing into two-cell condition in the cotton rat. In addition to the present data, we have observed by chance two 4-cell giant embryos in the Chinese hamster and one morula and one blastocyst in the rat which all developed normally as their sibs did (unpublished data). In sea urchins, as well as in some other invertebrate species, giant eggs have been known to be capable of developing into viable youngs (Wilson, 1928). In an European frog, *Rana esculenta*, triploid embryos derived from giant diploid eggs were shown to grow faster and larger than normal diploids and consequently to metamorphose into giant juvenile frogs (Uzzell *et al*., 1975; Berger and Uzzell, 1977). It is likely that the nuclear and cytoplasmic contents in giant diploid eggs are favorable for embryogenesis, at least for preimplantation stages in mammals, although their ultimate fate can not be assumed.

Based on a mathematical method for maximum likelihood analysis (Jacobs and Morton, 1977), Jacobs *et al*. (1978) estimated the causal mechanism of the compiled data with 48 cases of triploids of man in which the parental origin of the extra genome was investigated by the use of heteromorphic chromosome markers. According to them, all the digynic triploids were interpreted to be caused by the suppression of the first polar body emission. Their interpretation, however, raises a question especially related to the findings of the present study, since a considerable proportion of giant diploid eggs should have the same varieties of chromosomal complements as those of the eggs having failed the first meiotic division. Their analysis utilizing the mathematical method was strongly influenced by the preoccupation that the fertilization of a diploid gamete derived from

![Fig. 5. A chromosome preparation of a giant triploid two-cell zygote showing 33 chromosomes in each metaphase plate. The arrow indicates the tail of a fertilized spermatozoon.](image-url)
a tetraploid gonial cell is not a biologically plausible phenomenon. Thus, they excluded by mistake the possible contribution of giant diploid ova to human triploids.

In conclusion, it is emphasized that the giant diploid oocytes possibly contribute to the production of human digynic triploids, since various evidences concerning the giant diploid eggs clearly indicate the possibility of their development into digynic triploids in wide varieties of species including mammals.

Summary. The giant eggs twice the normal size in the Chinese hamster were studied cytogenetically with 2 primary and 18 secondary oocytes and 6 one-cell and 5 two-cell zygotes, in order to understand their biological significance as a cause of digynic triploidy. These oocytes exhibited respectively 22 tetrads and 22 dyads with normal morphology, indicating that meiotic chromosomal segregation had taken place normally. The developing giant eggs were all digynic triploids, and the frequency distribution of their two sex variants was 5 cases of XXX and 6 cases of XXY, giving a ratio of nearly 1:1, as expected. Importance of giant diploid oocytes was emphasized as a possible origin of human digynic triploidy.

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References