77. Chromosomal Anomalies Caused by Preovulatory Overripeness of the Primary Oocyte

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Since Mikamo (1961) first described a teratogenic effect of preovulatory overripeness of the primary oocyte on the embryonic development in *Xenopus*, it has been recognized that the overripeness of oocytes causes developmental and possibly chromosomal anomalies (Witschi and Laguens, 1963; Fugo and Butcher, 1966; Butcher and Fugo, 1967; Mikamo, 1968a, b). Recently Mikamo and Hamaguchi (1975) confirmed a highly significant increase of diandric triploidy in 1-cell rat embryos developed from the eggs which were aged in follicles by artificially delayed ovulation. Furthermore, they found an increase of aneuploids and mosaics in preimplantation blastocysts after the same treatment, although their samples were not large enough in number to prove statistical significance. Even in such an early developmental stage a great number of embryos of the delayed ovulation group were not karyologically analyzable due to declined mitotic activity. This fact suggests that earlier developmental stages may be more suitable in examining the chromosomal anomalies, and therefore, in clarifying their causal mechanisms.

In the present study we investigated the effect of delayed ovulation cytogenetically in 2-cell rat embryos.

**Materials and methods.** Sexually mature female rats (Wistar-Imamichi strain) were kept under a controlled light condition of 14 hr light (5:00 a.m.–7:00 p.m.) and 10 hr darkness for at least 3 weeks. The estrous cycles were recorded by daily vaginal smears, and animals with consistent 4-day estrous cycle were used in the experiment.

Ovulation was delayed for 48 hr by injections with pentobarbital sodium (30 mg/kg body weight) two days successively (once at 1:45 p.m. on the day of proestrus, and twice at 1:00 and 1:45 p.m. on the second day). On the third day, treated females were mated in the evening, and successful copulation was judged by the presence of a vaginal plug or of spermatozoa in the vaginal smear the following morning.

Two-cell embryos were collected from the oviducts by flushing about 52 hr after fertilization. After an observation of morphological
features under a dissecting microscope, the embryos were incubated with Eagle's MEM containing 30% fetal calf serum and 0.06 µg/ml colcemid for about 6 hr at 37°C. Chromosome slides were prepared with the method described by us previously (Kamiguchi et al., 1976, 1978). This method is available for analyzing two separate metaphase plates of a 2-cell embryo and for detecting a tail of a fertilized spermatozoon.

**Results and discussion.** The effects of delayed ovulation on fertilization and embryonic development are shown in Table I. The rate of fertilization and that of recovery of eggs from oviducts were decreased obviously in the experimental group (P<0.001). The latter suggests that some of the ova might have degenerated within the follicles without being ovulated. In fact, some corpora lutea in the experimental animals maintained follicular antrum and occasionally a degenerating ovum even 3 days after mating.

An increase of various developmental anomalies was also highly significant in the experimental group (P<0.001). The anomalies caused by delayed ovulation included the embryos delaying in development (1-cell) and 2-cell embryos with various abnormal features, such as degenerating blastomere(s), an extremely large polar body and unequal-sized blastomeres (Fig. 1). A great majority of these abnormal embryos did not attain metaphase of the second cleavage. Therefore, chromosome analysis was carried out mainly in morphologically normal 2-cell embryos.

A significant increase of chromosome anomalies in the delayed ovulation group was confirmed using a rather small number of specimens (Table II). Both aneuploids and mosaics increased significantly (P<0.05). Five aneuploids (3 monosomes and 2 trisomies) were observed in 278 control embryos, whereas 14 aneuploids (9 monosomes and 5 trisomies) in 267 treated embryos. Similarly, 5 mosaics (1 case of 42/43, 1 case of 41/42, 2 cases of 41/43 and 1 case of 39/45) were found in the control group, while 13 mosaics (4 cases of 42/43, 3 cases

**Table I. Effect of delayed ovulation on fertilization and embryonic development of the rat**

<table>
<thead>
<tr>
<th>Estrous cycle</th>
<th>No. of litters</th>
<th>No. of C. L. (mean)</th>
<th>No. of embryos collected (%)</th>
<th>No. of normal embryos (%)</th>
<th>No. of abnormal embryos (%)</th>
<th>No. of unfertilized eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (4-day)</td>
<td>38</td>
<td>590 (15.3)</td>
<td>550</td>
<td>515</td>
<td>36</td>
<td>9</td>
</tr>
<tr>
<td>Delayed (6-day)</td>
<td>72</td>
<td>1165 (15.3)</td>
<td>872</td>
<td>622</td>
<td>132</td>
<td>118</td>
</tr>
</tbody>
</table>

χ²-test: P<0.001  P<0.001  P<0.001  P<0.001
of 41/42, 5 cases of 41/43 and 1 case of 39/42) in the delayed ovulation group.

The mechanism to cause such mosaics with 42/43 chromosome constitution is very likely to be the anaphase lag which has taken place during the first cleavage division in trisomic zygotes. Since these trisomies must have been caused by nondisjunction during the first (MI) or the second meiotic division (MII) either in oogenesis or in spermatogenesis, these particular cases may be the result of two successive events of abnormal chromosome behavior, i.e. nondisjunction in meiosis and anaphase lag in the 1st cleavage. Total events of the abnormal chromosome behaviors were compared between the control and the experimental groups (Table III). The incidence was significantly higher in the experimental group than in the control group, both in the meiotic stages and in the 1st cleavage stage (P<0.01 and P<0.05, respectively). Thus, delayed ovulation was proven to induce abnormal behavior of meiotic and/or mitotic chromosomes, consequently, aneuploids and mosaics.

Other types of chromosome anomaly were haploidy and poly-
ploidy. A haploid embryo found in the experimental group had only an accessory spermatozoon in perivitelline space. This suggests that the spermatozoon might have activated the ovum but have not taken a part in any further fertilization process, accordingly the egg developed parthenogenetically.

Table III. Incidence of nondisjunction and anaphase lag

<table>
<thead>
<tr>
<th>Estrous cycle</th>
<th>No. of embryos analyzed</th>
<th>Sum of nondisjunction and anaphase lag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MI or MII* 1st cleavage Total</td>
</tr>
<tr>
<td>Normal (4-day)</td>
<td>278</td>
<td>6 5 11</td>
</tr>
<tr>
<td>Delayed (6-day)</td>
<td>267</td>
<td>18 13 31</td>
</tr>
<tr>
<td>χ²-test</td>
<td></td>
<td>P&lt;0.01 P&lt;0.05 P&lt;0.001</td>
</tr>
</tbody>
</table>

* The incidence may include not only the events occurred during oogenesis but also those during spermatogenesis.

A significant increase of polyploids was found in the delayed ovulation group (P<0.05). There were 6 cases of polyploids (6 triploids) in the control group, while 15 polyploids (12 triploids, 2 tetraploids and 1 pentaploid) in the experimental group. It is noteworthy that all polyploids except one found in the two groups were polyspermic (Fig. 2). This exceptional one was proven to be a digynic triploid derived from a giant ovum with the diploid number of chromosomes. Thus, Mikamo and Hamaguchi's finding (1975) that polyploids caused by delayed ovulation were almost exclusively polyspermy was clearly confirmed in this study. As they pointed out, it is quite possible that the polyspermy which occurred frequently in the aged ovum is due to the destruction of the defensive mechanism(s) which is provided in egg surface and/or zona pellucida against polyspermic fertilization. However, we do not ignore the possibility that a dense population of spermatozoon within the fertilization site of oviducts might have taken an important role in inducing the polyploids.

Summary. Effects of preovulatory overripeness of primary oocytes were studied cytogenetically in 2-cell rat embryos which developed from the ova unduely aged for 48 hours in mature follicles. Both aneuploids and mosaics increased significantly in the experimental group. It was clearly indicated that the delayed ovulation induces nondisjunction and anaphase lag of chromosomes during both the meiotic and first cleavage divisions. A significant increase of polyploidy was also proven as the result of the delay in ovulation. Its causal mechanism was confirmed to be polyspermy.

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References