**Table 1.** Development of (BALB/c ×C57BL/6) $F_1$ and ICR mouse eggs fertilized and cultured *in vitro*

| | No. and developmental rate[1] of embryos at each cleavage stage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1-cell | | 2-cell (24 h)[2] | | 4-cell (48 h)[2] | | Morula (72 h)[2] | | Blastocyst (96 h)[2] |
| $F_1$ | 103 | (85%) | 87 | (99%)* | 86 | (94%) | 81 | (99%)* | 80 |
| ICR | 141 | (77%) | 108 | (62%) | 67 | (85%) | 57 | (56%) | 32 |

1) Percentage of embryos that developed in each interval of cleavage stages. 2) Examination time in hours after the beginning of insemination. *Significant difference ($P<0.001$) from the corresponding value in ICR.

**Table 2.** Fertilization rate and incidence of first-cleavage mitosis in (BALB/c × C57BL/6) $F_1$ and ICR mouse eggs fertilized *in vitro*

| | $F_1$ | ICR |
|---|---|---|
| No. of eggs prepared | 565 | 680 |
| fertilized (fertilization rate) | 499(88.3%) | 627(92.2%)* |
| at pronuclear stage | 48( 9.6%) | 153(24.4%)*** |
| with 2 pronuclei | 15(31.2%) | 96(62.7%)*** |
| with 3 or more pronuclei | 33(68.8%) | 57(37.3%)*** |
| in mitosis | 451(90.4%) | 474(75.6%)*** |
| pre-syngamy[1] | 407(90.2%) | 453(95.6%)** |
| syngamy[2] | 44( 9.8%) | 21( 4.4%)** |
| unfertilized | 66(11.7%) | 53( 7.8%)* |
| in mitosis[3] | 4( 6.1%) | 3( 5.7%) |

1) The late prometaphase at which male and female haploid groups of chromosomes are able to be distinguished. 2) The metaphase at which 2 haploid chromosome groups have fused. 3) A haploid set of chromosomes showing parthenogenetic cleavage division. Significant difference from the corresponding value in $F_1$:*, $P<0.02$; **, $P<0.005$; ***, $P<0.001$.

ICR. The developmental rate was again reduced significantly ($P<0.001$) in ICR between the morula and the blastocyst stages.

Table 2 shows the differences between $F_1$ and ICR eggs in the fertilization rate and the incidence of first-cleavage mitosis. In chromosome preparations, successful fertilization could be determined by the presence of 2 or more haploid complements of chromosomes or pronuclei in the egg. The fertilization rate, expressed as the percentage of the number of fertilized eggs to eggs prepared, was significantly lower ($P<0.02$) in $F_1$ (499/565; 88.3%) than in ICR (627/680; 92.2%) eggs. Of fertilized eggs, a significantly higher proportion of eggs was in the pronuclear stage in $F_1$ than in ICR

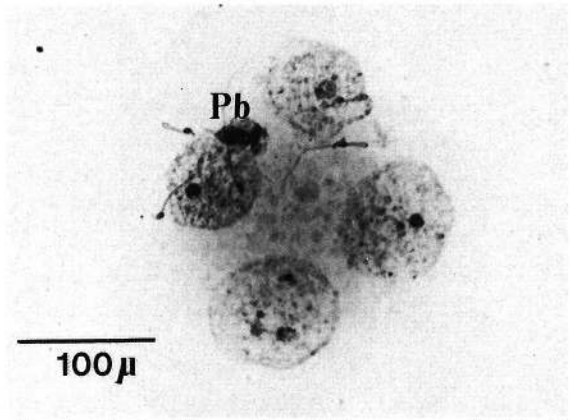

**Fig. 1.** A polyploid figure of an ICR mouse egg fertilized *in vitro*. Four pronuclei, 4 supplemental spermatozoa and a polar body (Pb) are observed.

eggs ($P<0.001$). The percentage of polyploid eggs (Fig. 1) with 3 or more pronuclei in the pronuclear stage was significantly higher in $F_1$ (68.8%) than in ICR (37.3%) ($P<0.001$). A highly significant difference between $F_1$ (90.4%) eggs and ICR eggs (75.6%) ($P<0.001$) in the proportion of eggs showing mitotic figures was obtained.

The mitotic eggs were in 2 distinct stages: The

**Table 3.** Incidence of chromosomal aberrations at first-cleavage stage in (BALB/c × C57BL/6) $F_1$ and ICR mouse eggs fertilized *in vitro*

| | $F_1$ | ICR |
|---|---|---|
| No. of eggs in mitosis | 455 | 477 |
| with analyzable chromosomes | 442(97.1%) | 454(95.2%) |
| 2n | 364(82.4%) | 416(91.6%)* |
| 2n+1 | 1( 0.2%) | 3( 0.7%) |
| 2n−1 | 4( 0.9%) | 6( 1.3%) |
| 3n | 69(15.6%) | 26( 5.7%)* |
| $n^{1)}$ | 4( 0.9%) | 3( 0.7%) |
| with structural aberration | 5( 1.1%) | 6( 1.3%) |

1) Unfertilized eggs listed in Table 2. *, Significant difference from the corresponding value in $F_1$ at $P<0.001$.

**Fig. 2.** A normal diploid figure of an ICR mouse egg fertilized *in vitro*. Two complete haploid groups of chromosomes in the "pre-syngamy" stage of first-cleavage mitosis and observed. The presence of the Y-chromosome (arrow) in the right group shows this embryo to be male. C-band staining after air-drying.

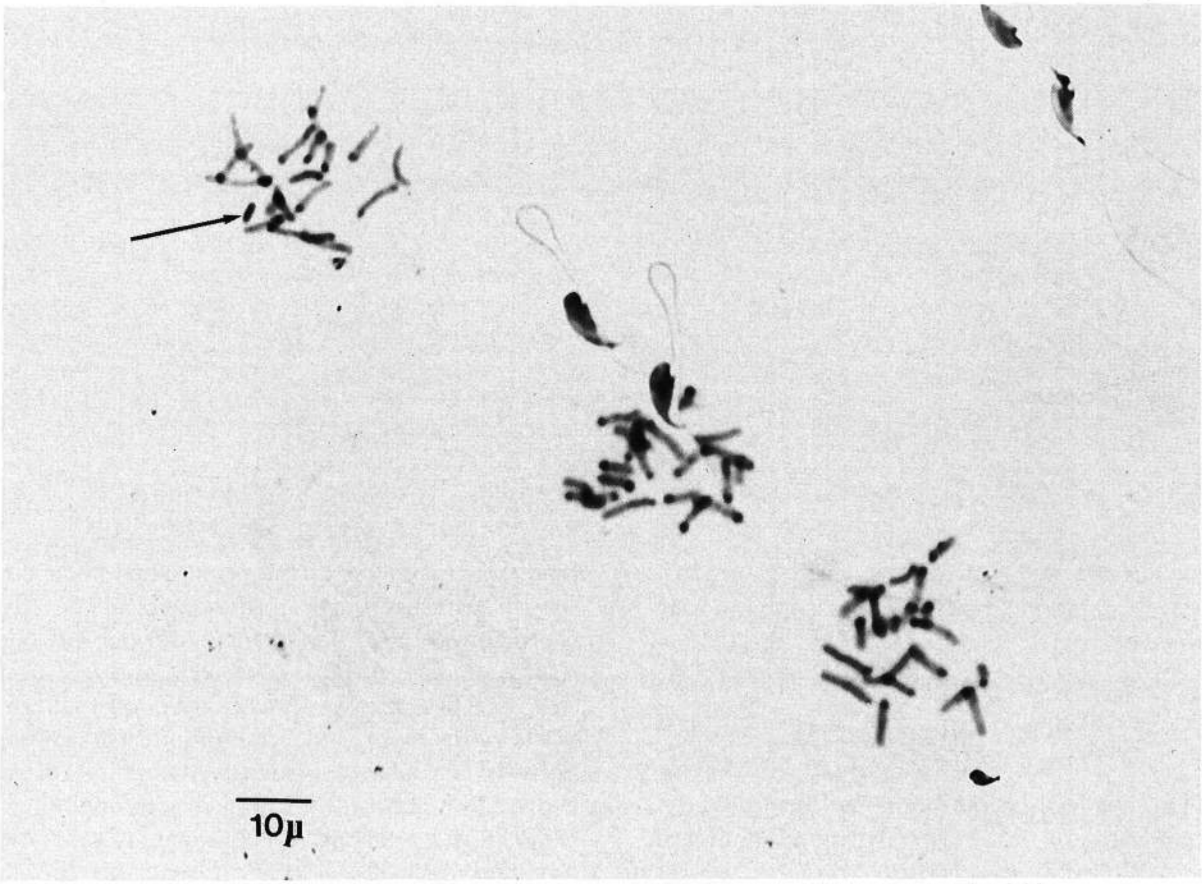

**Fig. 3.** A triploid figure of an $F_1$ mouse egg fertilized *in vitro*. Three complete haploid groups of chromosomes at the "pre-syngamy" stage of first-cleavage mitosis are observed. The presence of the Y-chromosome (arrow) in the top group shows the sex chromosome combination to be XXY. C-band staining after air-drying.

late prometaphase or "pre-syngamy" stage in which the maternal and paternal haploid chromosome groups appeared separately, and the metaphase or "syngamy" stage in which the chromosome groups had already fused. (The terminology is largely in accordance with that of McGaughey and Chang [10] and Kaufman [11]). The incidence of these subdivision is higher in ICR than in $F_1$ for "pre-syngamy" and lower in ICR than in $F_1$ for "syngamy" ($P<0.005$). These findings show that the first cleavage in $F_1$ eggs progresses faster than in ICR. A few eggs had only a haploid set of chromosomes, and they were defined as unfertilized ones.

Table 3 shows the results of the chromosomal analysis of mitotic eggs in $F_1$ and ICR. A normal diploid figure of ICR mouse eggs and a triploid figure of $F_1$ mouse egg fertilized *in vitro* are shown in Figures 2 and 3, respectively. The incidence of diploid was significantly higher in ICR and that of triploid was significantly lower in $F_1$ ($P<0.001$). No difference was found between $F_1$ and ICR eggs in the incidence of aneuploidy and haploidy. The incidence of haploidy and that of eggs with structural aberrations of chromosomes, such as gap, break and fragment were not significantly different in eggs from 2 strains.

Chromosomal sexing was made in the normal diploid (Fig. 2), hyperdiploid and triploid eggs, with a successful sexing rate of 97.7% (424/434) in $F_1$ and 98.9% (440/445) in ICR. As shown in Table 4, the sex ratio of diploid eggs (containing hyperdiploid ones) in $F_1$ and ICR was not statistically significant. The triploid eggs had different numbers of Y chromosomes (0, 1 or 2) among the 60 complement of chromosomes, indicating their sex chromosome combinations to be XXX, XXY or XYY. The frequencies of the combinations were in the order 17, 32 and 14 in $F_1$ and 5, 12, and 7 in ICR. These frequencies could be reduced to a