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Aneuploidy in reconstructed oocytes

Inability of mature oocytes to create functional haploid genomes from somatic cell nuclei

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Chromosomes of oocytes reconstructed by transferring cumulus cell nuclei into enucleated mouse oocytes were examined. No oocytes had a normal haploid complement of chromosomes after emission of a pseudo-polar body.

Several investigators have reported that nuclei of adult somatic cells undergo meiosis-like divisions after incorporation into maturing and mature oocytes, and suggested that the nuclei thus produced could substitute for male or female gamete nuclei for treating human infertility (1-5). The segregation of somatic chromosomes into two groups within maturing oocytes was reported previously by others (6), but correct assortment of chromosome homologues was not shown.

Mature oocytes were collected from oviducts of superovulated (B6D2)F_1 females. Oocytes were enucleated and injected with single nuclei from mouse (2n = 40) or Chinese hamster (2n = 22) cumulus cells as described (7). Two to three hours after nucleus injection, oocytes were activated by two-hour treatment with 5 mM $SrCl_2$ in Ca^{2+}-free CZB medium, followed by four-hour incubation in regular CZB medium without Sr^{2+} at 37˚C under 5% CO_2 in air. The oocytes that emitted a pseudo-polar body (ppb) were cultured in CZB medium containing 0.01µg/ml vinblastine for 13-14 hours to enforce arrest at the metaphase of the first mitotic division, and then fixed and air-dried for chromosome examination (8). Some oocytes were immunocytochemically stained to observe morphology of spindle before the emission of a ppb.

The number of mouse cumulus chromosomes that remained in activated oocytes after ppb extrusion was less than or greater than the expected haploid number of 20 chromosomes in 153 of 168 cases (91.1%) (Figure 1A). In 4.2% of the constructs, all 40 chromosomes remained in the oocyte. In 1.2% of the constructs, no chromosomes remained in the oocyte. The number of chromosomes segregated to the oocyte showed a mode of 20 and a mean of 20 $\pm$ 8 S.D. Immunofluorescence staining demonstrated that the single chromatids of the somatic nucleus were randomly arranged on the spindle prior to ppb extrusion (Figure 1B). To determine whether 8.9% of oocytes could segregate homologous chromosomes from cumulus cell nuclei, we produced additional constructs by injecting Chinese hamster cumulus cell nuclei. Unlike mouse chromosomes, individual Chinese hamster chromosomes are readily identifiable (9). The modal number of Chinese hamster chromosomes retained in the mouse oocyte was 10, with a mean of 12 $\pm$ 3

S.D. Only 18 of the 128 hamster-mouse constructs (14.1%) retained 11 chromosomes - the expected haploid number of chromosomes in the hamster, and upon karyotyping none had a normal haploid complement of chromosomes.

The above results clearly show that authentic haploid nuclei are not generated in activated oocytes following somatic cell nuclear transfer. This result is not surprising since, in the absence of normal prophase events of meiosis (i.e. synapsis and recombination between homologous chromosomes), there is no physical means of ensuring the orderly segregation of homologous chromosomes.

Assuming random segregation of homologous chromosomes, the probability of achieving correct chromosome assortment can be calculated by a statistical formula, ${}^{2^r}/_{n}C_r$, where *n* and *r* are diploid and haploid chromosome numbers, respectively. If the chance of obtaining oocytes with a haploid number of chromosomes is taken as 8.9% in the mouse (2n=40) and 14.1% in the Chinese hamster (2n=22), the chance of correct haploidization is approximately 6.79×10^{-7} in the former and 4.09×10^{-4} in the latter. Since human diploid chromosome number is 46, it is certain that the chance of correct haploidization in humans is less than that in the mouse. Further, even if haploidization were to occur, it is unlikely that the resulting group of chromosomes would provide a functionally normal haploid complement.

Normal embryo development requires appropriate genomic imprinting of both parental genomes (10). Even if a normal haploid number of chromosomes were to be retained within the oocyte, it is extremely unlikely that such a chromosome complement would, by simple assortment, contain exclusively maternally or paternally imprinted chromosomes, as would be needed to substitute for authentic gamete genomes. One might propose that imprints could be erased and re-established in this system. However, given the stage-specificity of the imprinting process it is unlikely that metaphase II oocytes would contain factors needed to erase imprints and re-establish maternal imprints. There is clearly no reason to suppose that an oocyte of any stage could establish a paternal system of imprints. In short, the proposed use of

somatic cell nuclear transfer into mature oocytes for the purpose of creating functional haploid genomes appears untenable.

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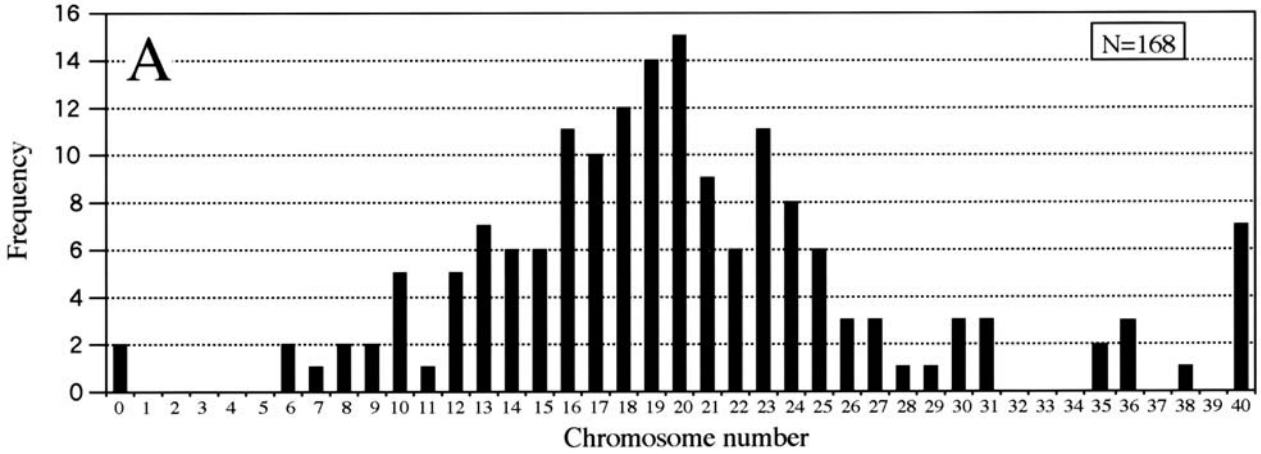
Figure Legends

Figure 1

(A) Variation in number of mouse cumulus chromosomes remaining in the oocyte after the emission of a pseudo-polar body. N is the total number of oocytes examined.

(B) Fluorescence microscopy of an oocyte incubated for 2-3 hr after enucleation followed by injection of a mouse cumulus cell nucleus. Panel a: Immunocytochemical staining of spindle. Panel b: DAPI staining of chromosomes. Spindle microtubules are aberrantly assembled and chromosomes are distributed disorderly.


A
N=168
Frequency
Chromosome number
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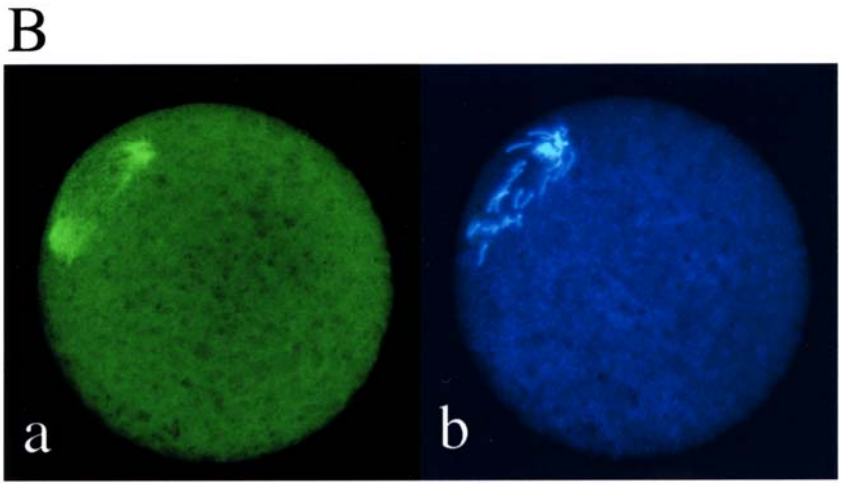
0 2 4 6 8 10 12 14 16


B
a
b