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# Factors Affecting Meiotic and Developmental Competence of Primary Spermatocyte Nuclei Injected into Mouse Oocytes[1]

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## ABSTRACT

**Mature mouse oocytes that have received the nuclei of pachytene primary spermatocytes (or metaphase I chromosomes of primary spermatocytes) can develop into fertile offspring. However, success rate in this study was low. No more than 3.8% of transferred 2-cell embryos arising from spermatocyte-injected oocytes developed to full term. Nevertheless, the birth of normal offspring seems to suggest that at least in some primary spermatocytes the functional genomic imprinting is complete before transfer and/or consolidated after the transfer. Although injected spermatocyte nuclei could undergo two successive meiotic divisions within oocytes, abnormalities of both divisions were commonly observed, and sister chromatids often separated prematurely during the second meiotic division. Chromosome breakage/rearrangements were also frequently seen before the first cleavage. Such abnormalities of chromosome behavior are probably the major causes of the poor preimplantation development of zygotes arising from primary spermatocyte-injected oocytes. Thus, clinical use of primary spermatocytes as substitutes for spermatozoa in assisted fertilization is not advisable until the causes of chromosomal abnormalities are better understood through extensive animal studies.**

## INTRODUCTION

In most vertebrates, female gametes become ready for fertilization at the metaphase of the second meiotic division [1, 2]. In contrast, male gametes complete two successive meiotic divisions, then transform into motile spermatozoa before becoming competent for fertilization. Under normal conditions, motionless haploid spermatids cannot fertilize even when they are brought directly onto the surface of the oocytes. We have shown previously that the nuclei of round spermatids can participate in embryonic development if they are transferred microsurgically into oocyte cytoplasm [3, 4]. Nuclei of secondary spermatocytes can also support development when they are allowed to complete the second meiotic division within oocytes [5]. We have reported that primary spermatocyte nuclei undergo two successive meiotic divisions within a single maturing oocyte [6, 7] or within two fully mature oocytes [7], and some develop into full-term offspring [7]. Recently, Ogura et al. [8] reported the birth of fertile offspring following electro-fusion of maturing mouse oocytes with the primary spermatocytes. Here we also report the development of fertile mice from oocytes receiving the nuclei/chromosomes of the primary spermatocytes. However, we also report a high incidence of chromosome abnormalities in meiotic division of injected spermatocytes, which may account for the low success rate of the method and suggests the step-wise requirement for developmental acquisition of competence by spermatocytes to undergo the meiotic divisions.

## MATERIALS AND METHODS

### *Reagents and Media*

All inorganic and organic reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless stated specifically. The medium for culturing oocytes after microsurgery was bicarbonate-buffered Chatot, Ziomek, Bavister (CZB) medium supplemented with 5.56 mM D-glucose and 4 mg/ml BSA [9, 10]. The medium for oocyte collection and microsurgery was a modified CZB medium with 20 mM Hepes-HCl, 5 mM $NaHCO_3$, and 0.1 mg/ml polyvinyl alcohol (cold water-soluble, $M_r$ 360 000) in place of BSA. The original CZB and Hepes-CZB media [9, 10] contained 0.1 mM EDTA. We eliminated EDTA from our media because we had proved some lots of EDTA to be toxic to mouse oocytes/zygotes. CZB and Hepes-CZB were used under 5% $CO_2$ + 95% air and 100% air, respectively. The pH of these media was approximately 7.4.

### *Preparation of Mature Oocytes*

B6D2F1 (C57BL/6 female × DBA/2 male) female mice, 8–12 wk old, were superovulated by consecutive i.p. injections of 5 IU eCG and 5 IU hCG 48 h apart. Fully mature oocytes were collected from oviducts between 14 and 17 h after hCG injection. They were freed from cumulus cells by treatment for 2–3 min with 0.1% bovine testicular hyaluronidase (300 USP U/mg; ICN Pharmaceutical, Costa Mesa, CA) in Hepes-CZB. Cumulus-free oocytes were rinsed thoroughly and kept in CZB for up to 3 h under 5% $CO_2$ in air.

### *Isolation and Injection of Primary Spermatocytes*

The methods used for isolation of primary spermatocytes and their subsequent injection into oocytes were basically the same as those described previously for secondary [5] and primary spermatocytes [7], but spermatocyte isolation and injection were carried out at room temperature (24–27°C) rather than at the lower temperatures recommended by Kimura and Yanagimachi [11]. Briefly, seminiferous tubules, collected from 7- to 14-wk-old B6D2F1 male mice, were put in Hepes-CZB and cut into small pieces with a pair of fine scissors. A drop of the above medium with tubule fragments was mixed with the same volume of Hepes-CZB containing 12% (w:v) polyvinylpyrrolidone (av. $M_r$ 360 000) and pipetted vigorously to release spermatogenic cells (and spermatozoa) from the tubules. The largest

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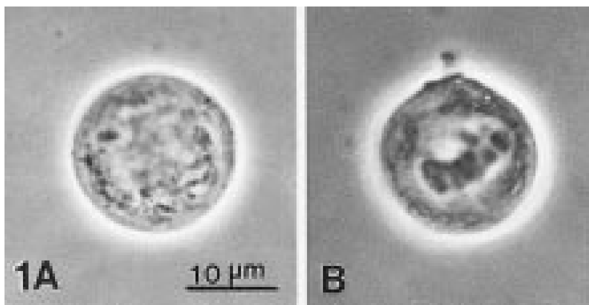


FIG. 1. Phase-contrast micrographs of a pachytene primary spermatocyte (**A**) and a spermatocyte at the first meiotic metaphase (**B**).

mononuclear cells (18–20 μm in diameter, Fig. 1A) were judged as the primary spermatocytes at mid- or late-pachytene stages (and perhaps a few at the diplotene stage). This was confirmed by injecting these cells into oocytes and examining their homologously paired meiotic chromosomes (cf. Fig. 4 of reference [5]). The large cells (about 20 μm in diameter) with a metaphase spindle (Fig. 1B) were judged to be primary spermatocytes at the metaphase of the first meiotic division (Met-I spermatocyte). A single pachytene (or Met-I) spermatocyte was drawn in and out of an injection pipette (7–8 μm diameter) until the plasma membrane was broken. The nucleus or chromosomes, with or without accompanying cytoplasm, were injected immediately into a mature oocyte. When incubated for 80 min to 3 h at 37.5°C, two metaphase spindles were seen within one oocyte. One of the spindles was larger than the other. Chromosome analyses revealed that the larger spindle-chromosome complex was of primary spermatocyte origin [7]. When such oocytes were activated by 30-min treatment with $Ca^{2+}$-free CZB containing 5 mM $SrCl_2$ [12, 13] and examined 3–6 h later, the majority had two polar bodies and two pronuclei. One polar body was larger than the other. Similarly, one pronucleus was larger than the other. These larger polar bodies and pronuclei were presumed to be of primary spermatocyte origin [7]. The larger polar body was aspirated into the injection pipette. Its delicate plasma membrane was broken during aspiration. The nucleus, with or without accompanying cytoplasm, was injected immediately into another mature oocyte. After 90-min to 2-h incubation in CZB at 37.5°C, the oocytes were activated with the $Sr^{2+}$ medium and examined 5–7 h later. Eggs with two polar bodies and two pronuclei were considered normally fertilized and were cultured further. We conducted these experiments with two slightly different protocols. In the first (protocol I), the inner diameter of the injection pipette for handling polar bodies was 7–9 μm, while it was 9–10 μm in the second (protocol II). The time interval between primary spermatocyte injection and the onset of $Sr^{2+}$-treatment varied between 80 min and 180 min in protocol I, while it was limited to 80–90 min in protocol II. In protocol I, the $Sr^{2+}$ medium for oocyte activation was freshly prepared and kept in a refrigerator for 1–7 days before use, while in protocol II only freshly prepared $Sr^{2+}$ medium was used. About 100 spermatocyte-injected oocytes were fixed and stained by the whole-mount method for cytological details [14]. Some were fixed and spread on slides for examination of individual chromosomes [15, 16]. About 20 oocytes were cultured in the presence of 0.006 μg/ml vinblastin to arrest them at the metaphase of the first cleavage for examination of chromosomes.

### *Embryo Transfer*

Two-cell embryos, 18–24 h postactivation, were transferred into oviducts of pseudopregnant CD1 (albino) females that had been mated the previous night to vasectomized males of the same strain. Nineteen days after transfer, the females were killed and their uteri were examined for the presence of live fetuses. Live fetuses, if any, were collected by Caesarian section and raised by other lactating CD1 females.

### *Animals*

Animals used in this study were maintained in accordance with the guidelines of the Laboratory Animal Service at the University of Hawaii and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources National Research Council (DHEW publication no. [NIH] 80–23, revised in 1985). The protocol of our animal handling and treatment was reviewed and approved by the Animal Care and Use Committee at the University of Hawaii.

## RESULTS

Figure 2 shows the behavior of nuclei of late pachytene spermatocytes after injection into mature unfertilized oocytes. An injected nucleus underwent a premature chromosome condensation (Fig. 2, A and B) while the oocyte remained unactivated (arrested at Met-II). By 80 min after injection, chromosomes presumed to be of primary spermatocyte origin were arranged on a metaphase spindle (arrow in Fig. 2C). They look similar in their gross appearance to those of Met-I chromosomes of the oocyte itself (Fig. 2C inset). When the oocytes were activated by $Sr^{2+}$, chromosomes on two metaphase spindles were segregated, but with some lagging on one spindle (arrows in Fig. 2D). Lagging chromosomes were seen in half of 10 oocytes examined around 50 min after onset of $Sr^{2+}$-treatment. When examined 5–6 h after oocyte activation, two sets of pronucleus-polar body were found, one being larger than the other. The larger one (arrows, Fig. 2E) was presumed to be of primary spermatocyte origin, and the smaller one to be derived from the oocyte. When the nucleus of the larger polar body was injected into another unfertilized oocyte, it underwent a premature chromosome condensation (Fig. 2, F and G). By 80 min after injection, two ''identical'' metaphase chromosome-spindle complexes were seen within one oocyte (Fig. 2H). When such oocytes were activated by $Sr^{2+}$-treatment, chromosomes were segregated (Fig. 2I) and two identical sets of pronucleus-polar body were formed (Fig. 2J). In a series of experiments, a total of 1009 oocytes received injections of pachytene spermatocytes. Eighty-three percent survived injection, and the majority of surviving eggs had two polar bodies and two pronuclei after $Sr^{2+}$-treatment (Table 1). Similar results were obtained when 602 oocytes received injections of Met-I spermatocyte chromosomes (Table 1).

When the nuclei of larger polar bodies, presumed to be of primary spermatocyte origin, were injected into other mature oocytes, two identical metaphase chromosome-spindle complexes were seen in each oocyte, as already stated (Fig. 2H). Close examination of these oocytes after fixation and staining by the whole-mount method revealed that chromosomes in one group (most probably, oocyte chromosomes) were arranged in an orderly fashion on the metaphase plate (Fig. 3A), whereas those in another group (per-

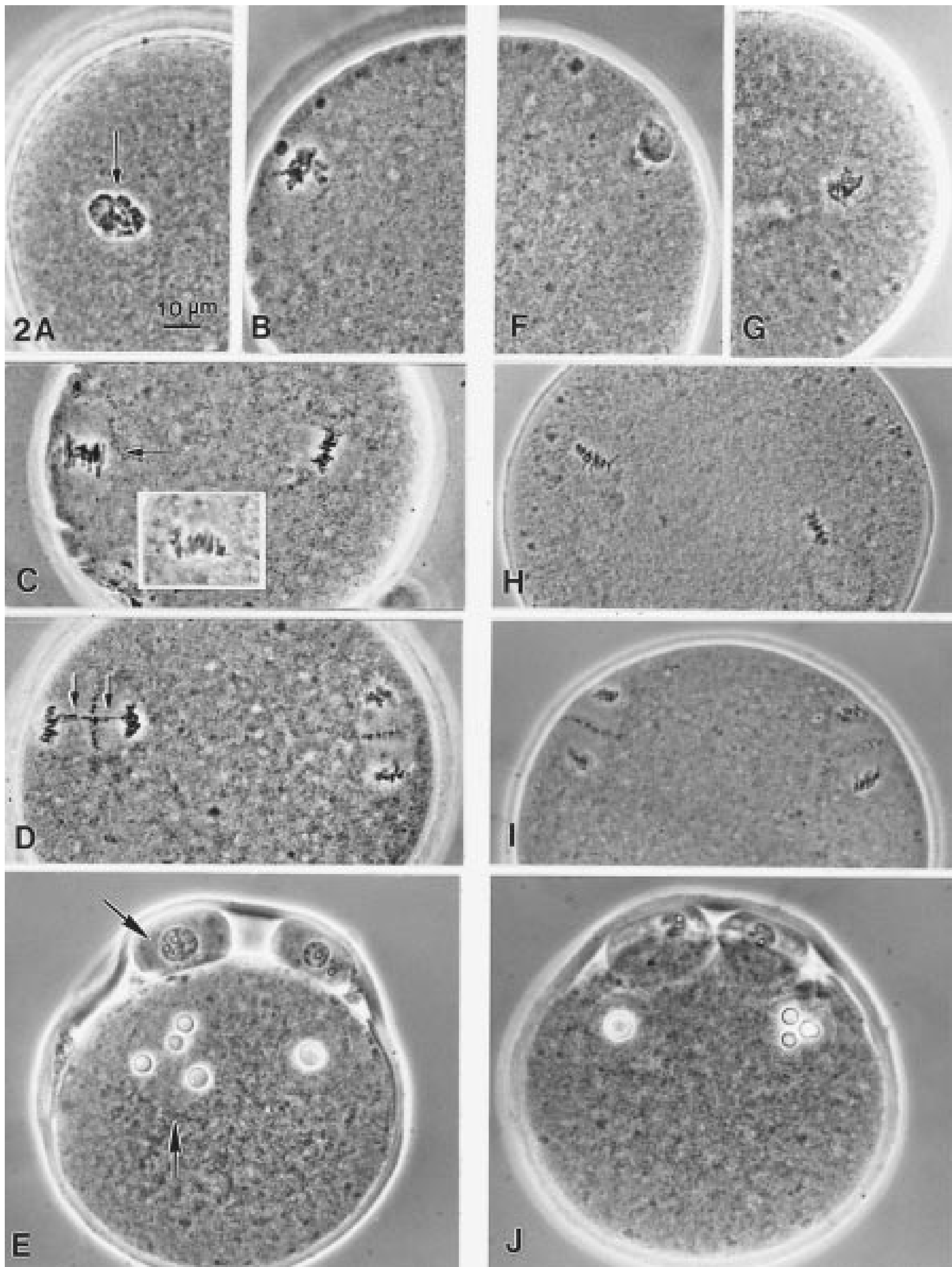


FIG. 2. Mature oocytes that received injections of pachytene spermatocyte nuclei. **A**) Spermatocyte nucleus (arrow) soon after injection into a mature oocyte. **B**) About 30 min after injection: nucleus is undergoing premature chromosome condensation. **C**) About 80 min after injection: chromosomes presumed to be of spermatocyte origin (arrow) are arranged on metaphase plate; another metaphase spindle is presumed to be of the oocyte itself. Inset shows Met-I chromosomes of a maturing oocytes; note that spermatocyte Met-I and oocyte Met-I are similar in their appearance. **D**) About 20 min after 30-min $Sr^{2+}$-treatment: the oocyte is activated, and sister chromatids are segregated except a few lagging ones (arrows). **E**) About 4 h after $Sr^{2+}$-treatment: larger polar body and larger pronucleus (arrows) are presumed to be of spermatocyte origin. **F**) Another mature oocyte that received polar body nucleus of spermatocyte origin, immediately after injection. **G**) About 30 min after injection: the nucleus is undergoing chromosome condensation. **H**) About 80 min after injection: two sets of metaphase chromosomes are seen in this oocyte. **I**) About 20 min after $Sr^{2+}$-treatment: two anaphase spindles are seen. **J**) About 4 h after $Sr^{2+}$-treatment: two polar bodies and two pronuclei are seen. One pair is presumed to be of spermatocyte origin, the other of the oocyte itself.

TABLE 1. Status of oocytes receiving injections of pachytene primary spermatocytes (Pach) or metaphase 1 spermatocytes (Met-1), examined 3-5 hours after $Sr^{2+}$ activation.[a]

| Spermatocyte injected | Total no. of oocytes receiving injections (exp. no.) | No. (%) of oocytes surviving | No. (%) of surviving oocytes with[b]: | | |
|---|---|---|---|---|---|
| | | | 2Pb + 2PN | 1Pb + 3PN | Others |
| Pach | 1009 (29) | 836 (83) | 702 (84) | 74 (9) | 60 (7)[c] |
| Met-I | 602 (24) | 487 (81) | 405 (83) | 45 (9) | 37 (8)[d] |

[a] Experiments were performed using protocol I (see *Materials and Methods*).
[b] Abbreviations: 1Pb and 2Pb, one and two polar body (-ies), respectively; 2PN and 3PN, two and three pronuclei, respectively; first polar bodies were not included here.
[c] These include twenty-five unactivated oocytes, ten 4PN oocytes, and eighteen 1Pb + 1PN oocytes.
[d] These include eleven unactivated oocytes, eight 4PN oocytes, and four 2PN + 1PN oocytes.

TABLE 2. Status of oocytes receiving injections of polar body nuclei (PbN) of presumed primary spermatocyte origin, examined about 6 h after $Sr^{2+}$ activation.[a]

| Origin of PbN | Total no. of oocytes receiving PbN (exp. no.) | No. (%) of surviving oocytes | No. (%) of surviving oocytes with[b]: | | |
|---|---|---|---|---|---|
| | | | 2Pb + 1PN | 1Pb + 3PN | Others |
| Pachytene | 658 (29) | 519 (79) | 394 (76) | 80 (15) | 45 (9)[c] |
| Met-I | 393 (24) | 314 (80) | 242 (77) | 37 (12) | 35 (11)[d] |

[a] Experiments were performed using protocol I (see *Materials and Methods*).
[b] Abbreviations: same as Table 1.
[c] These include sixteen unactivated oocytes, nine 4PN oocytes, and eighteen 2PB + 1PN oocytes.
[d] These include seven unactivated oocytes, seven 4PN oocytes, and two 2PB + 1PN oocytes.

haps spermatocyte chromosomes) were less regularly arranged (Fig. 3B). Such imprecision in chromosome arrangement was seen in 50% of 30 oocytes examined. When spread chromosomes were examined, chromosomes in one group (most probably those of oocyte) were clearly in the configuration of Met-II chromosomes with sister chromatids attached at centromeres (Fig. 3A′). In the other group, fewer chromosomes exhibited the typical Met-II morphology (Fig. 3B′), and, instead, premature sister chromatid separation was evident in 66% of 29 analyzable oocytes.

When 1051 oocytes received injections of first polar body nuclei presumed to be of primary spermatocyte or of Met-I spermatocyte origin, the majority survived the operation (Table 2). When activated by $Sr^{2+}$, the majority had two identical sets of polar bodies and pronuclei (Fig. 2J, Table 2). When 80 randomly selected pronuclear (2Pb + 2PN) eggs were cultured in vitro, the majority developed to the 4-cell stage, but only a few reached the blastocyst stage (Table 3). Examination of chromosomes of eggs arrested at the metaphase of the first cleavage by vinblastin revealed that chromosomes in one group (most probably those of female pronucleus origin) were normal in number (n = 20) and structure (Fig. 4A), whereas those in the other group (most likely those of male pronucleus origin) were

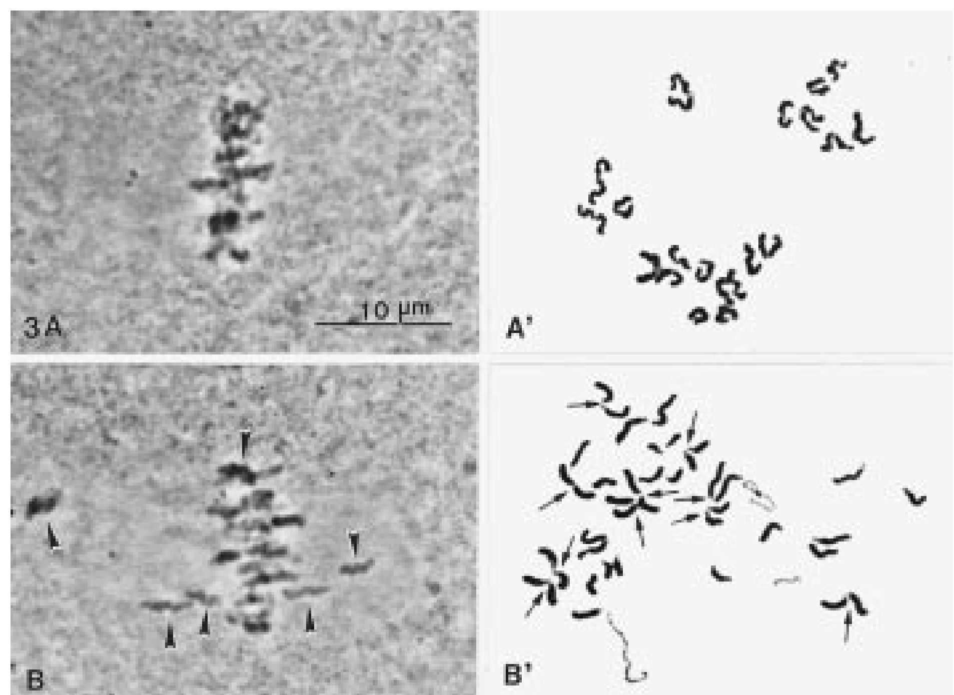


FIG. 3. Chromosomes in mature oocytes that received polar body nuclei of primary spermatocyte origin. These oocytes correspond to the oocytes shown in Figure 2H. **A**) Presumed oocyte metaphase II chromosomes, side view, whole-mount preparation: all chromosomes are arranged on the metaphase plate. **A′**) Air-dried preparation of chromosomes corresponding to **A**: all chromosomes are of typical Met-II configuration. **B**) Presumed spermatocyte chromosomes at Met II, side view, whole-mount preparation: some chromosomes (arrowheads) are away from the metaphase plate. **B′**) Air-dried preparation of chromosomes corresponding to **B**: note that many sister chromatids are separated, although some (arrows) are attached at centromere.

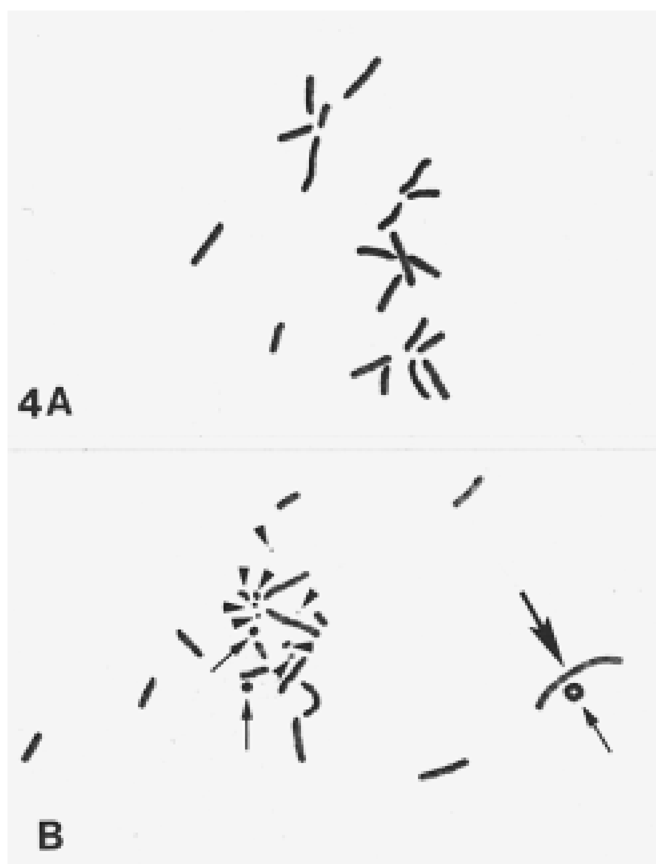


FIG. 4. Chromosome spread of an egg arrested at metaphase of the first cleavage. **A**) Chromosomes presumed to be of oocyte origin (n = 20). **B**) Chromosomes presumed to be of spermatocyte origin, where many chromosomes are broken or fused/exchanged. One translocation (large arrow), three rings (small arrows), and eight fragments (arrowheads) are seen.

abnormal in most cases (Fig. 4B). Only one of 12 eggs examined had structurally and numerically normal chromosomes in both chromosome groups.

A total of 590 normal-looking two-cell embryos were transferred to 57 foster mothers. Of these, 9 embryos reached full term. Five died (or were cannibalized by mothers) shortly after birth or within a week. Others (3 females and 1 male) survived and reached maturity (Table 4). These four mice as well as their offspring (Fig. 5) all proved to be fertile; litter sizes were also all normal (5–11).

## DISCUSSION

In this study we provide information about the behavior and developmental success of primary spermatocyte nuclei injected into oocytes. First, we show the birth of viable and fertile mice from spermatocyte-injected oocytes. Second,

TABLE 3. In vitro development of zygotes produced by injection of polar body nuclei (PbN presumed of primary spermatocyte origin) into mature oocytes.[a]

| Origin of PbN | No. of zygotes cultured (exp. no.) | No. (%) of zygotes developed to: | | | |
|---|---|---|---|---|---|
| | | 2-cell | 4-cell | Morula | Blastocyst |
| Pachytene | 44 (3) | 44 (100) | 36 (81) | 16 (36) | 2 (5) |
| Met-I | 36 (5) | 35 (97) | 31 (86) | 21 (58) | 2 (6) |

[a] Experiments were performed using protocol I.

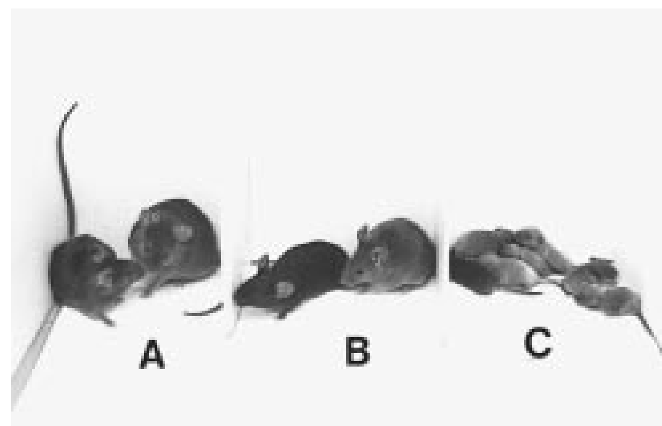


FIG. 5. Male and female mice developed from oocytes that received spermatocyte nuclei (**A**) and their F1 (**B**) and F2 (**C**) offspring.

we provide additional observations confirming prior evidence [6, 7] that spermatocyte nuclei can undergo meiotic divisions inside the oocyte. Thus it seems likely that common factors drive cell cycle progression in both the oocytes and spermatocytes, a conclusion bolstered by previous observations on metaphase-promoting factor activation in spermatocytes induced to enter metaphase [17, 18]. Third, we provide new details about the meiotic success of injected spermatocyte nuclei and the behavior of spermatocyte chromosomes in the oocyte. These data give insight into a puzzling observation: in spite of the ability of spermatocyte nuclei to undergo the mechanics of meiotic division in oocytes, there was chromosome damage (Fig. 4), and the success rate of obtaining live offspring from oocytes receiving injected spermatocyte nuclei was very low. At best, only 3.8% of the two-cell embryos from oocytes receiving spermatocyte injections reached full term (Table 4). Ogura et al. [8] also could obtain only 3 full-term offspring from 90 preimplantation embryos produced by fusion of maturing oocytes with primary spermatocytes. The high incidence of chromosomal abnormalities and the low frequency of normal embryonic development as reported in this study may be due to technical issues and/or may have a biological explanation. Thus the two important issues addressed here are technical aspects of nuclear transfer and the acquisition of biological competence of spermatocyte nuclei for meiotic divisions.

The abnormalities in chromosome behavior and the low developmental success of injected oocytes may be due to technical issues. Clearly, practice and experience improve these demanding techniques. When the birth of mice after injection of round spermatids into mature oocytes was first reported, the success rate was only 1.7% [3]. The rate has

TABLE 4. Result of embryo transfer.

| Exp. protocol[a] | Spermatocyte injected | No. of embryos transferred (no. of recipients) | No. of term fetuses | No. of offspring reaching maturity[b] |
|---|---|---|---|---|
| I | Pachytene | 302 (29) | 1* | 0 |
| II | Pachytene | 104 (8) | 4* | 1M,1F |
| I | Met-I | 184 (20) | 4 | 2F |

[a] See *Materials and Methods.*
[b] M, male; F, female.
* Differences are statistically significant (Fisher's exact probability test, $p = 0.016$).

increased with technical improvements [4, 19–22], so that recently, 40–50% of mouse oocytes receiving injections of round spermatids developed into normal offspring (unpublished data), only slightly less than that (60–70%) achieved after injection of immature testicular spermatozoa or mature epididymal spermatozoa. The most likely source of mechanical damage to spermatocytes and their chromosomes is the injection procedure. We found (Table 4) that embryonic development was better when we used an injection pipette with a larger inner diameter (9–10 μm, in protocol II, rather than the smaller, 7–9 μm pipette used in protocol I). We have recently had good developmental success in transferring the oocyte polar body by these methods [23] in an experiment that is in some aspects a ''control'' for those reported here. However, we found that the nuclei of the polar bodies of presumed spermatocyte origin were especially vulnerable to mechanical shear and stress, perhaps accounting for some of the chromosome breaks observed. Thus, clearly, mechanical aspects of the transfer procedure can be improved, and this could lead to greater developmental success.

The poor rate of developmental success of primary spermatocyte-injected oocytes may also have its roots in the biology of spermatocyte and oocyte maturation. We know a great deal about the step-wise development of nuclear competence for meiotic division by oocytes [24], but until recently there has been no experimental means to assess acquisition of competence for meiotic division in spermatocytes. In vitro culture and microinjection provide experimental approaches to the problem. Wiltshire et al. [17] cultured mouse primary spermatocytes and induced premature entry into Met-I by treatment with okadaic acid. The condensed Met-I chromosomes were bivalent (never univalent) and had normal numbers of chiasmata. Thus competence to condense bivalents held together by chiasmata arises long before the spermatocytes normally enter the meiotic division phase. However, these conditions did not support the anaphase transition, so it was not possible to determine whether spermatocytes had also acquired competence for normal chromosome segregation.

The present study assessed spermatocyte competence for chromosome segregation by injecting spermatocyte nuclei into oocytes, which can provide the structural machinery (spindle) for completion of meiotic divisions. Although spermatocyte nuclei were competent to respond to anaphase signals, there were abnormalities in behavior of the spermatocyte chromosomes. The chromosomes did attach to the spindle at metaphase (see Fig. 2C, arrow); however, at anaphase, chromosome lagging and failure to separate homologous chromosomes in an orderly manner was seen frequently (Fig. 2D, arrow). Additionally, observations of the spermatocyte Met-II chromosomes revealed that premature sister chromatid separation occurred before the second meiotic division. These chromosome anomalies could arise if the spermatocyte was not yet competent for meiotic chromosome segregation (for example, recombination might not be complete) and/or if the ooplasm failed to provide some factor necessary for spermatocyte chromosome segregation. In this regard, it is important to remember that, while the oocyte meiotic divisions occur long after recombination and desynapsis of homologous chromosomes, the spermatocyte meiotic divisions occur immediately after recombination and are associated with desynapsis of the paired homologous chromosomes. Moens and coworkers [25, 26] have demonstrated redistribution of synaptonemal complex proteins as spermatocytes enter the first meiotic division. Although this redistribution also occurs in cultured spermatocytes induced prematurely to enter the division phase [27], it is not known if it can occur normally in the ooplasm. If it does not, the result could be anomalous segregation of chromosomes and premature sister-chromatid segregation, as we have observed in some injected oocytes.

Taken together, our observations suggest the possibility that most primary spermatocytes have not acquired the competence for normal chromosome segregation and/or that the ooplasm does not provide adequate factors required to segregate the spermatocyte chromosomes that are still synapsed. Interestingly, the abnormalities in attachment of spermatocyte chromosomes to the spindle and their segregation did not appear to trigger a checkpoint arresting the cell division process as might be expected. This is consistent with recent evidence that oocytes lack chromosome-mediated checkpoint mechanisms [28].

Other biological mechanisms may also influence the outcome of oocytes receiving spermatocyte injection, although these issues were not directly assessed in this study. Differential imprinting of the maternal and paternal genomes are required for normal development [29–34]. It is not known exactly when the paternal imprint is established [29–34], but developmental success of some spermatocyte-injected oocytes suggests that the functional imprint may be established before the meiotic divisions. Another important issue relevant to these experiments is the DNA repair capacity of gametocytes. Mouse pachytene spermatocytes have DNA repair mechanisms [35], but it is not known if this activity is present once the nucleus is removed from the spermatocyte cytoplasm. This is especially relevant if the spermatocyte chromosomes are damaged by either transfer procedure or by inadequate segregation in the oocyte. Oocytes have the ability to repair DNA damage in the nuclei of fertilizing spermatozoa and, indeed, there are strain-specific differences in this repair capacity [36]. Perhaps the oocyte's DNA repair capabilities are overwhelmed by damage in the injected spermatocytes, or possibly greater success in normal development could be obtained by selecting oocyte recipients high in DNA repair capacity.

In spite of a high frequency of abnormalities in meiotic chromosome behavior, apparently a few spermatocyte nuclei were capable of completing meiosis normally and supporting normal development. Since it is difficult to distinguish definitively diplotene nuclei from pachytene nuclei under the conditions we employed, and since diplotene nuclei are present at low frequency, it is interesting to speculate that the successful development derived from late meiotic prophase nuclei that had achieved full competence to undergo accurate meiotic chromosome development and support development. Definitive proof of this awaits both absolute identification of the stage of the successful spermatocyte nuclei and genetic proof that the offspring contain two genomes, one spermatocyte-derived and one oocyte-derived. Our experimental protocol made use of hybrid B6D2F1 oocytes and spermatozoa because of the overall robust success of these germ cells in culture conditions. We consider that it is highly unlikely that the offspring produced were derived from anything other than the injection of spermatocyte chromosomes. One might imagine that the polar body nuclear transfer in some cases might have resulted in the transfer of an oocyte chromosome into another oocyte; nevertheless, this would not be expected to give rise to offspring since it is well known that gynogenetic

and parthenogenetic embryos do not develop to term due to imprinting effects [29, 30].

The results reported here document not only abnormal chromosome behavior in the many primary spermatocytes injected into oocytes but also the successful development of offspring from some oocytes that received injections of spermatocyte nuclei. This mixture of good news and bad news raises a cautionary note about the use of immature germ cells for immediate clinical purposes. The kinds of anomalies we observed in chromosome behavior are precisely those that give rise to aneusomic gametes and aneuploid conceptuses, ensuring lethality or developmental abnormalities. In any consideration of extending this technique to humans, it must be remembered that the consequences of autosomal aneuploidy in the mouse are preterm lethalities, but in the human, some autosomal aneuploidies (trisomy 13,18,21) are viable to term and subsequently, but with severe medical problems. Most importantly, these results raise issues about the step-wise acquisition of spermatocyte competence for meiotic division and the ability of the oocyte to provide factors needed for the segregation of synapsed spermatocyte chromosomes. The experimental system we have reported provides a means to analyze these interesting and important problems and thus to learn more about the mechanics of meiosis as well as about the developmental competence of male germ cells.

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