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The Control of Mammalian Female Meiosis: Factors that Influence Chromosome Segregation

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INTRODUCTION

Species survival depends on the consistent production of normal, viable gametes. Evolutionary pressure, therefore, should ensure that the error rate in gametogenic cell divisions remains low. Indeed, in the few eukaryotic species that have been studied in detail, the frequency of abnormal gametes resulting from errors in meiotic chromosome segregation is extremely low (Table I). The human, however, is a notable exception: an estimated 10–25% of all human conceptions are chromosomally abnormal due to an error in chromosome segregation during meiosis (e.g., Refs. 1–3). The vast majority of the errors are incompatible with survival and result in demise of the conception during the first trimester.

The combination of a high meiotic error rate and a low tolerance for alterations in chromosome dosage sounds like a recipe for evolutionary disaster. Although assisted reproduction and preimplantation genetic diagnosis may be increasingly important in human reproduction in coming generations, research efforts should not be directed solely toward waging evolutionary warfare. An understanding of how and why meiotic errors occur in our species is of fundamental importance and may provide better long-term options for circumventing reproductive problems in our species.

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The development of molecular and molecular cytogenetic techniques has provided new tools for analyzing the meiotic process. These techniques not only provide improved methods for the detection of chromosomally abnormal human embryos, but also provide new tools for gaining insight to the mechanisms responsible for the high meiotic error rate in our species. This manuscript summarizes recent studies of the factors that influence mammalian meiotic chromosome segregation and presents new hypotheses to explain the high error rate in human female meiosis. Many important questions remain unanswered, and where possible, I have attempted to identify some of the important areas for future research.

IN THE HUMAN FEMALE, MEIOTIC CHROMOSOME SEGREGATION IS ERROR-PRONE AND STRONGLY INFLUENCED BY AGE

The development of molecular techniques for the identification of DNA polymorphisms made possible investigations of the parent and meiotic stage of origin of chromosomally abnormal human conceptions. The combined data from these studies demonstrate that greater than 95% of human aneuploidy is attributable to errors in female meiosis (4). The difference in the error rate between spermatogenesis and oogenesis has been further characterized in direct studies of human oocytes and sperm using fluorescence in situ hybridization (FISH). These studies have uniformly demonstrated a low error rate (of the order of 0.1%/chromosome, with the overall rate of aneuploidy estimated at 1–2%) in sperm (reviewed in Refs. 5 and 6). In contrast, although the estimates from studies of oocytes have been quite variable, it is likely that the error rate for human female meiosis is an order of magnitude higher than that for the male (for recent reviews see Refs. 1, 2, 5, and 7).

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Table I. The Frequency of Meiotic Chromosome Errors[a]

Yeast	<1%
C. elegans	<1%
Female *Drosophila*	<1%
Female mouse	1–2%
Human female	10–25%

[a] For lower eukaryotes, estimates were derived based on chromosome-specific error rates from the following sources: yeast (55), *C. elegans* (56), *Drosophila* (17). For the mouse and human, estimates are from Refs. 57 and 3, respectively.

Variation in aneuploidy estimates from studies of human oocytes undoubtedly reflects, at least in part, differences in study populations. The frequency of meiotic chromosome errors is strongly influenced by maternal age, hence the incidence of meiotic errors is dependent on the age structure of the study population. The complex nature of the maternal age effect can most easily be appreciated by examining the data from the study of human trisomies, those conceptions with an additional chromosome (reviewed in Ref. 6). As shown in Fig. 1, the incidence of trisomic conceptions does not show a linear increase with age. Rather, the incidence remains fairly constant until the later stages of the reproductive life span of the human female, when the incidence of trisomy sharply increases.

The basis of the maternal age effect remains unknown and the study of age-related changes in the human oocyte is complicated by the fact that the process of meiosis is initiated prenatally but not completed until after the oocyte has been fertilized. All oocytes enter meiosis during fetal development and remain suspended in prophase of the first meiotic division until just prior to ovulation. Hence, completion of the first meiotic division may take 40 years or longer. The protracted nature of the division has led to speculation that the age-related increase in nondisjunction may be the result of damage accrued during the arrest period (8,9). Alternatively, the basis of the age effect has been postulated to be due to events occurring prenatally (10) or during the periovulatory period (e.g., Refs. 11–16). Although the basis of the age effect remains unknown, as described in the following two sections, recent studies have provided important new data regarding the effect of age on the meiotic process.

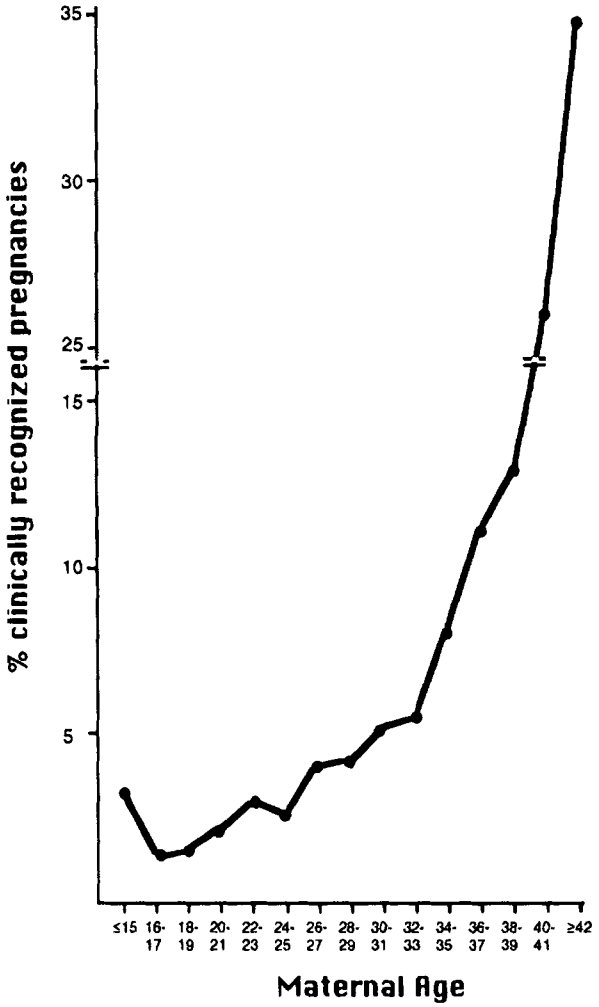

Fig. 1. The incidence of trisomy among clinically recognized pregnancies. (Reproduced with permission from *Annual Reviews Inc.* and based on data from Ref. 58.)

HUMAN NONDISJUNCTION IS ASSOCIATED WITH ALTERED MEIOTIC RECOMBINATION

At the first meiotic division the number of chromosomes is reduced by half. This is accomplished via a mechanism of chromosome segregation unique to the first meiotic division whereby centromeres of homologous chromosomes segregate to opposite spindle poles (Fig. 2). During prophase of the first meiotic division, homologous chromosomes undergo a process of pairing and genetic exchange (recombination). The sites of exchange play a key role in the segregation of homologs at the first meiotic division (reviewed in Refs. 17 and 18). As the chromosomes condense in preparation for division, the sites of exchange become visible as chiasmata, physical associations between the arms of homologous chromosomes. Chiasmata are believed to assist in maintaining the associated homologues in an orientation that promotes the attachment of their centromeres to opposite spindle poles. Once a pair of homologues has formed attachments to opposite poles, the site(s) of exchange maintain a physical con-

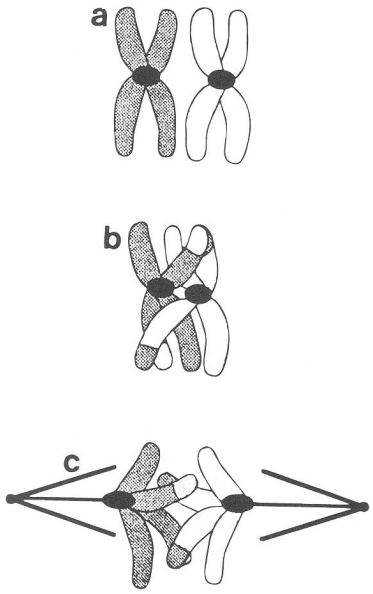

Fig. 2. Chromosome behavior during the first meiotic division. (a) Homologous chromosome pair. (b) Exchange or recombination events are initiated. (c) At metaphase, the sites of exchange (chiasmata) counterbalance the forces directing centromeres to opposite poles.

nection between homologues, acting to counterbalance the forces pulling the centromeres to opposite poles and, thus, allowing the chromosome pair to orient at the spindle equator. Proper orientation on the spindle at metaphase, in turn, acts to facilitate the appropriate segregation of the homologues at anaphase. Thus, chiasmata, the physical connections that result from recombination, function to ensure proper chromosome segregation at the first meiotic division.

The importance of genetic recombination in the segregation of chromosomes during meiosis has been recognized from the study of recombination deficient mutants in experimental organisms. Mutants that decrease or abolish recombination have been described in both yeast and female *Drosophila,* and all such mutants show a corresponding increase in the incidence of meiotic chromosome nondisjunction. In addition, recombination is the only factor, other than maternal age, that is clearly associated with meiotic nondisjunction in the human. Genetic maps generated for human trisomies involving 15, 16, 18, 21, and the X chromosome all show a significant reduction in length by comparison with comparable genetic maps generated for normal conceptions (reviewed in Ref. 19). Because map length is a measure of genetic exchange, these studies suggest that a reduction in recombination is associated with aberrant meiotic chromosome segregation. This implies that, in the human female as in lower eukaryotes, a reduction in recombination results in a corresponding increase in meiotic chromosome segregation errors.

Exciting new data, however, suggest that the effect of recombination on chromosome segregation is not quite so simple: recently, it has become obvious that, in organisms as evolutionarily diverse as *Drosophila* and humans, the *placement* of the exchange events on the chromosome arms can substantially alter the fidelity of meiotic chromosome segregation (19). Chiasmata positioned either too close to the centromere or too close to the ends of the chromosome increase the likelihood of meiotic segregation errors in both species (9,20,21). Furthermore, in the human female, where the incidence of meiotic errors is extraordinarily high and strongly influenced by maternal age, these aberrant sites of recombination are associated with chromosome segregation errors that occur at both the first and the second meiotic division (21). These findings have led to the proposal of a "two-hit" hypothesis to explain meiotic nondisjunction in the human female (21). According to this hypothesis, the first hit occurs during the prenatal period when the process of recombination is initiated and susceptible exchange configurations are established. The second hit, acting at the time of reinitiation and completion of the first meiotic division, further increases the likelihood of a segregation error in homologues with susceptible exchange configurations. The mechanism associated with the second hit remains unknown but is hypothesized to become more frequent with increasing maternal age, thus accounting for the maternal age effect on meiotic chromosome segregation.

EVIDENCE THAT FEMALE MEIOSIS LACKS AN IMPORTANT CHECKPOINT CONTROL MECHANISM

In addition to recombination, the fidelity of chromosome segregation is ensured by cell cycle control mechanisms that regulate transition points in the cell cycle. Cell cycle checkpoint control mechanisms function to detect mistakes and inhibit cell cycle progression under conditions that would result in grave errors in the cell division process (reviewed in Refs. 22–24). Of particular interest from the standpoint of chromosome segregation is the checkpoint mechanism that regulates anaphase onset. This metaphase/anaphase transition checkpoint has been described in both mitotic and nonmammalian meiotic cells. It has been

described as a spindle assembly or a chromosome-mediated checkpoint, because defects in either spindle formation (reviewed in Ref. 25) or the alignment of chromosomes at metaphase (e.g., Refs. 26–29) can delay the onset of anaphase. In some organisms, the failure of alignment of even a single chromosome is sufficient to prevent the cell from initiating anaphase (26,29).

By ensuring that all chromosomes are properly attached to the spindle and aligned at the spindle equator before anaphase is initiated, this cell cycle control mechanism functions to prevent the missegregation of chromosomes to daughter cells. Because mistakes in the segregation of chromosomes during meiosis have particularly disastrous consequences, it seems likely that the meiotic cell cycle should be subject to stringent metaphase/anaphase checkpoint control. However, recent studies in our laboratory suggest that this checkpoint is absent or only partially functional during mammalian female meiosis (30,31). In studies of oocytes from XO female mice, we demonstrated that the single X chromosome failed to make a stable bipolar attachment and align at the spindle equator in a substantial proportion of metaphase cells (30). Furthermore, the presence of the univalent X chromosome at the first meiotic division appeared to influence other chromosomes in the complement, resulting in an increased frequency of misalignment of autosomal chromosomes at both first and second meiotic metaphase. The frequency of autosomal aneuploidy is unusually high among preimplantation embryos from XO females (32), suggesting that the failure of chromosome alignment at metaphase results in segregation errors at anaphase. This, however, is contrary to dogma, because failure of one or more chromosomes to align at the spindle equator should trigger the chromosome-mediated checkpoint control mechanism, causing a delay in the onset of anaphase until proper chromosome alignment is achieved. To determine if we could detect a subset of cells that were delayed or arrested at metaphase I, we initiated cell cycle progression studies of oocytes from XO females and XX control siblings. We found no evidence of delay or arrest at metaphase I, suggesting that female meiosis lacks the normal checkpoint control mechanism that monitors chromosome alignment at metaphase (31).

The lack of metaphase/anaphase checkpoint control provides a biological explanation for the high incidence of meiotic errors in the human female. Furthermore, because the available evidence suggests that a stringent checkpoint mechanism operates during male meiosis (reviewed in Ref. 31), the lack of a comparable checkpoint in female meiosis may provide a reason for the difference in the error rate between oogenesis and spermatogenesis.

Given the importance of fidelity in meiotic cell division, the absence of this important cell cycle control mechanism in female meiosis is surprising. Indeed, a plausible explanation is that the cell cycle control machinery does exist but is operationally impaired due to the excessive volume of the mammalian oocyte. In sea urchin embryos, during the first few cleavage divisions, when the blastomere volume is still large, errors in chromosome alignment do not induce metaphase delay (33). If the same is true in mammals, then metaphase/anaphase checkpoint control may be nonfunctional during early cleavage divisions. This has important clinical implications because it could provide an explanation for the phenomenon of confined placental mosaicism and associated intrauterine growth retardation in human conceptions (34). Moreover, it may provide an explanation for the high incidence of mosaicism in human preimplantation embryos (e.g., Refs. 35 and 36). Finally, it raises a cautionary flag for human assisted reproduction. Because the early cleavage divisions of the human embryo take place *in vitro*, it is possible that spindle integrity and/or chromosome alignment could be compromised by minor alterations in the culture environment, thereby increasing the risk of mosaicism.

MEIOTIC DEFECTS IN HUMAN OOCYTES SUGGEST THAT ANEUPLOIDY MAY BE THE RESULT OF DEFECTS IN THE GROWTH PROCESS OF THE HUMAN OOCYTE

From studies of spontaneously aborted human conceptions, we have come to view the maternal age effect as an increase in segregation errors at anaphase that typically involves one homologous pair of chromosomes. The lack of checkpoint control observed in murine oocytes suggests that these errors reflect the inability of the oocyte to detect and correct misalignment of a homologous pair at metaphase. This may indeed be the case, but recent studies of human oocytes have also demonstrated striking age-related defects in the formation of the meiotic spindle and in metaphase chromosome alignment. The nature of these defects suggests a more severe decline in the quality of the human oocyte with age.

Indirect immunofluorescence studies of MII arrested oocytes in two laboratories have demonstrated defects

in spindle formation and chromosome alignment in the *majority* of oocytes obtained from reproductively aged donors (37,38). In contrast, similar abnormalities were observed in a minority of oocytes from donors under the age of 35 years. Although the population of oocytes studied was different [in vitro matured oocytes from unselected antral follicles obtained from unstimulated ovaries (38) and oocytes obtained from periovulatory follicles (37)], the observed defects and the age association were strikingly similar in the two studies.

These studies suggest that the age-related increase in meiotic nondisjunction in the human female may actually be one symptom of a more global decline in oocyte quality. We have interpreted the age-related defects in spindle formation and chromosome alignment, as evidence that the growth process of the human oocyte becomes compromised in the reproductively aged ovary (38). This hypothesis is based on the fact that the mammalian oocyte does not acquire the competence to resume and complete meiosis until the late stages of the growth process.

The oocyte initiates meiosis during the prenatal period but remains suspended in prophase until just prior to ovulation. In preparation for ovulation, the oocyte undergoes an intense period of growth during which the machinery and regulatory components necessary for the completion of meiosis, fertilization, and the first several mitotic cleavage divisions of the embryo are stockpiled in the oocyte. Prior to growth, the oocyte is incapable of meiotic resumption. Studies of oocytes obtained at successive stages of growth during the first wave of folliculogenesis in the murine ovary have demonstrated that the ability to resume and complete the first meiotic division is acquired by the oocyte in stepwise increments during the late stages of the growth process (e.g., Ref. 39). According to our hypothesis, the defects in meiotic spindle formation and chromosome alignment that characterize the majority of oocytes obtained from reproductively aged human donors are evidence of perturbations in the late stages of oocyte growth. Interestingly, in recent meiotic studies of a mouse mutant in which folliculogenesis is compromised and all oocytes are developmentally incompetent, we have observed strikingly similar meiotic defects in chromosome alignment and spindle formation (Hunt, in preparation). Moreover, by studying oocytes obtained from immature antral follicles of control females, we have been able to verify that the observed defects are not simply a feature of meiotic immaturity. That is, studies of the first meiotic spindle in partially competent murine oocytes (i.e., oocytes capable of resuming the first meiotic division and of progressing to metaphase but incapable of initiating anaphase) have provided no evidence of similar meiotic defects. These results suggest that the meiotic defect in the mouse mutant (and perhaps in oocytes from reproductively aged human donors as well) represent perturbations in the growth process and not simply incomplete oocyte growth.

According to this hypothesis, the increase in meiotic defects in oocytes from reproductively aged donors reflects a decline in the oocyte growth process. If this is indeed the case, then the development of a culture system to support the *in vitro* growth of human follicles may provide a means of obtaining better quality oocytes from women in the latter years of their reproductive lifespan. Moreover, because several different systems have been developed to support the *in vitro* growth of murine follicles (40–43), the mouse may prove to be a valuable model for studying factors that influence meiotic chromosome segregation.

THE FUTURE

The development of several new technologies makes the answers to important questions about meiotic chromosome behavior finally within our grasp. For example, the recent development of human artificial chromosomes (44) makes possible studies aimed at identifying the essential structural components necessary for the appropriate behavior and segregation of chromosomes during cell division. As our definition moves beyond a descriptive one and we begin to understand the essential components of a mammalian centromere and how they function, we will undoubtedly gain new insight to errors in chromosome segregation.

Similarly, in the past decade we have begun to unravel the complex interactions among the cell cycle engine, microtubules, microtubule motor proteins, and the chromosomes. The study of mutants that affect cell division in lower eukaryotes has resulted in the identification of some of the genes involved in these processes. More recently, the identification of mammalian homologs of these genes provides the first step toward understanding the role of individual genes in mammalian mitosis and meiosis. The second step, targeted mutagenesis in the mouse to create "knockouts" of the genes, has been accomplished for several important cell cycle genes, including *Mos* (45,46), *Atm* (47–49), and several of the DNA mismatch repair genes (50–54). The study of mice homozygous for these manmade mutations has revealed not only mitotic defects but also, in several cases, meiotic defects.

Detailed analysis of the meiotic process in male and female mutants will provide insight to the role of these genes in mammalian meiosis and will also allow the first systematic approach to understanding differences in the control of spermatogenesis and oogenesis.

Advances in assisted reproductive technology not only have improved the chances of a successful reproductive outcome for millions of couples, but also have provided methods of diagnosing genetic defects at the preimplantation stage. An important spinoff of this technology has been increased access to human oocytes and the first studies of blastomeres from human embryos. These studies have and will continue to provide new insight to the frequency and mechanisms of chromosome errors in our species.

Finally, the development of systems for the *in vitro* growth of immature murine oocytes (40–43) has pioneered the way for the development of similar technology in the human. The development of a culture system to support the *in vitro* growth of human follicles could make it possible to circumvent the costly and potentially dangerous stimulation procedures currently used to obtain human oocytes, provide a more effective means of oocyte banking, and perhaps provide a means of obtaining better quality oocytes from women in the latter years of their reproductive life span. Moreover, from a research perspective, defining the culture conditions necessary to support normal growth and development of immature oocytes provides a powerful new approach to understanding the factors that control and influence the meiotic process. Increased understanding of the normal process will no doubt provide insight to the age-related changes that result in the extraordinarily high frequency of errors in meiotic chromosome segregation in our species.

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