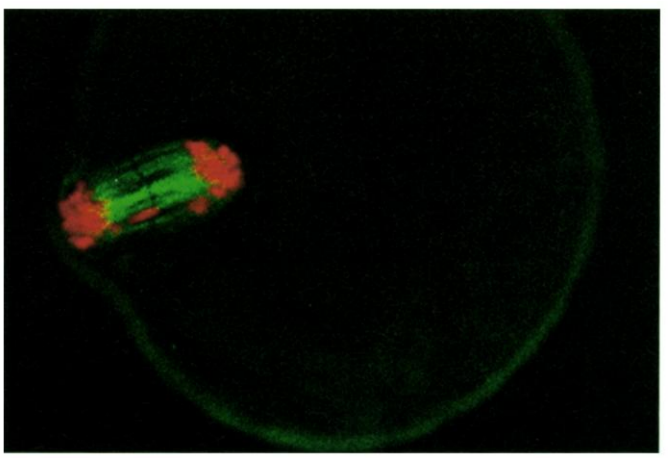
Sorting Out Chromosome Errors

Researchers are struggling to understand—and possibly overcome—aneuploidy, the most common cause of miscarriage

Humans are not particularly good at making babies. We have a flaw that becomes more pronounced as we age, and the limitation cannot be helped by candlelight and violins, Viagra, or, for the most part, fertility clinics. More often than in any other species, embryos we conceive have an abnormal number of chromosomes, a condition called aneuploidy. Most of the time, aneuploidy leads to miscarriage, but it is also responsible for Down syndrome and other mental and physical problems. "Aneuploidy is the most important problem in human reproduction," says Terry Hassold, a cytogeneticist at Case Western Reserve University in Cleveland, Ohio, who studies the many odd chromosomal configurations that humans create. "It's unquestionably the most common genetic problem our species has, and we don't have a clue why it happens."

Researchers might not have discovered why we conceive so many aneuploid offspring, but by studying embryos, eggs, and sperm in humans and other species, they have made solid progress in determining how, where, and when the process of distributing chromosomes to progeny goes awry. The advent of in vitro fertilization (IVF), which has high failure rates that appear strongly tied to aneuploidy, has also pushed the field forward. So, too, has the discovery that some chromosomes are more prone to aneuploidy than others. "There's been an extraordinary spurt in terms of interest and new knowledge about aneuploidy," says Hassold, who has been in the field for more than 25 years.

Nowhere is the spurt more tantalizing than in studies of the molecular machinery that drives meiosis, the cell division process that leaves eggs and sperm each holding half the total number of chromosomes—and the main source of aneuploidy (see p. 2181). "Until very recently, we didn't know what to look for," says Dorothy Warburton, a cytogeneticist at Columbia University in New York City and an authority on miscarriage. "As we begin to recognize the molecular players, there'll be obvious things to do." This applies both to basic researchers who are attempting to understand how meiosis stumbles, as well as fertility specialists.



Misdirected. The older the oocyte, the more likely its chromosomes (red) will get stuck during meiosis, possibly due to faulty spindles (green).

Three's a crowd

The first estimates of the frequency of aneuploidy came in a landmark 1975 study. French researchers Joëlle and André Boué, along with Philippe Lazar, analyzed the chromosomes in nearly 1500 first-trimester miscarriages. They found chromosomal abnormalities in a startling 61% of the samples. Of these, aneuploidies—usually three copies of the same chromosome or a single copy of the female sex chromosome—accounted for more than two-thirds of the abnormalities. Subsequent large studies showed slightly lower rates, leading to the now widely accepted view that chromosomal abnormalities explain half of all miscarriages, with aneuploidies accounting for roughly 60% of the problem. (Other relatively common abnormalities include three or more complete sets of chromosomes, called polyploidy, and "translocations," in which unmatched chromosomes combine with each other.)

All but a small percentage of aneuploidies end in miscarriage. The only trisomies that aren't lethal—those involving chromosome 13, 18, or 21 (Down syndrome), or sex chromosome combo XXY, XYY, or XXX—make up the single largest genetic cause of mental retardation and developmental disabilities. Warburton points out that chromosomes 13, 18, and 21 carry the fewest genes; trisomies of larger chromosomes are apparently so disruptive that they always cause miscarriage. Only one monosomy, X0, isn't lethal; it causes physical but not cognitive problems.

Aneuploidy occurs far more often in humans than in other species, says pathologist Kurt Benirschke, former director of the Center for Reproduction of Endangered Species at the San Diego Zoo. "But it's very rarely studied in other species," cautions Benirschke, now a professor emeritus at the University of California, San Diego. Aneuploidy in mice, the most intensively investigated mammal other than humans, occurs in no more than 2% of fertilized eggs.

In part because humans have such high rates of aneuploidy, miscarriage is staggeringly common. Allen Wilcox and colleagues at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina, stunned many people in 1988 when they reported in *The New England Journal of Medicine* what is still one of the most-cited estimates of human miscarriage rates. They asked 221 women who were attempting to become pregnant to collect urine samples each day. The researchers assessed some 30,000 samples for human chorionic gonadotropin, a hormone that spikes when an embryo implants in the uterus. They found that 31% of embryos miscarry after implantation begins.

The urine test does not account for other lost fertilizations that never make it to implantation. Many studies have attempted to peek through the window between conception and the week or so before implantation by assessing the chromosomes of embryos fertilized through IVF. Abnormalities run as high as 70%, with many embryos carrying a "mosaic" of both aneuploid and normal cells. Wilcox and others caution that IVF data do not necessarily reflect the general population: Most people seek IVF because they are having fertility problems; the embryos analyzed are often those left unused; and the technique itself might alter chromosomes. Still, says Wilcox, "IVF has added something to our complete ignorance" about the frequency of aneuploidy in the earliest stages of development.

Scrambled eggs

In the intensive hunt for the causes of aneuploidy, only one clear-cut risk factor has emerged: maternal age. But as fly geneticist R. Scott Hawley stresses, even that association is often misunderstood. "It's not how old the woman is," says Hawley, who studies aneuploidy at the Stowers Institute for Med-

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ical Research in Kansas City, Missouri. "It's how close she is to menopause."

Females make all of their eggs, or oocytes, when they are fetuses, and they are born with between 1 million and 2 million. Women go through menopause not when they reach a specific age, but when they have about 1000 oocytes left, most of the others having died through "atresia," a little-understood process of programmed cell death. The closer a woman is to menopause, the more frequently embryos formed from her eggs will have aneuploid chromosomes.

Many theories have attempted to explain why aging human oocytes are prone to aneuploidy. They share one underlying perspective: It's linked to how the eggs are made. Oocytes in a developing female fetus begin with two copies of each chromosome. Meiosis—a two-stage process of cell division referred to in shorthand as MI and MII—reduces the 46 chromosomes to 23. But, for some reason, shortly after MI begins, it arrests, effectively throwing the oocytes into a deep freeze. These oocytes will not "thaw" and reenter meiosis until they vie to become the one egg that is typically ovulated each month during a woman's reproductive years.

Arrest is a generalized feature of female meiosis in a range of species, from mammals to mollusks, says cytogeneticist Patricia Hunt of Case Western, although the evolutionary reason is unclear. But human eggs, with our comparatively long life-spans, face extra perils, because they have to wait as long as 50 years to complete their journey through MI and MII. "It strikes me as dangerous to do," says Hunt. As decades pass, oocytes have an increasingly difficult time separating their chromosomes into two even groups. Studies by Case Western's Hassold and others have shown that 90% of all chromosomal abnormalities have a maternal origin, and the vast majority of those trace back to an MI error in which chromosomes do not properly separate, or "disjoin."

There's more than one road to aneuploidy, but the field has long sizzled with debate about the mechanisms behind the most common pathways. In the once-popular "production line" theory, posited by University of Cambridge geneticists S. A. Henderson and Robert G. Edwards (the famed IVF pioneer) in 1968, the oocytes first produced by the fetus are more fit and are the first to be ovulated. As a woman approaches menopause, this theory holds, she has more and more "bad" eggs. The theory has since been disproven, but it helped lead to today's favored theory.

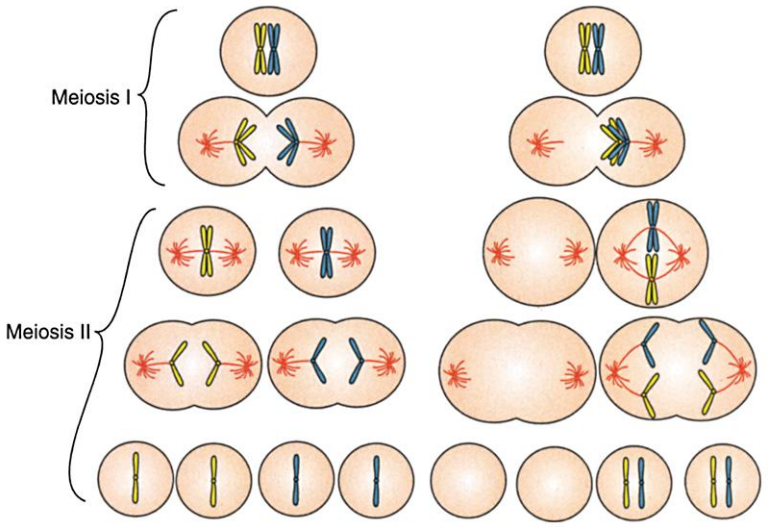
When MI begins, the maternally and paternally inherited copies of each chromosome connect to each other at places called chiasmata. This allows homologous chromosomes to, in essence, combine in their own version of sex and swap similar pieces of DNA. Henderson and Edwards found that mouse oocytes had fewer chiasmata as they aged, and the chiasmata they did have were more often located near the ends of the chromosomes. As a result, these chromosomes exchanged less DNA, a process known as recombination. Reduced or absent recombination thus served as a surrogate marker for the underlying problem: Poorly placed or absent chiasmata somehow gummed up disjunction.

Many experiments have verified Henderson and Edwards's suggestion that homologous chromosomes with few or unfortunately located chiasmata are prone to nondisjunction. But the team's other suggestion, which ties the maternal age effect to chiasmata becoming creakier over time, hasn't held up.

In 1996, two groups came up with a new theory to explain the discrepancy. Fly geneticist Hawley, then at the University of California, Davis, Kara Koehler, and co-workers studied nondisjunction in the X chromosome of *Drosophila melanogaster*. Neil Lamb and Stephanie Sherman of Emory University in Atlanta, Georgia, teamed up with other researchers, including Hassold, to examine nondisjunction in humans with Down syndrome. Both teams arrived at what's now known as the "two-hit" theory.

In hit number one, diminished recombination—caused by a lack of chiasma or their mislocation—creates "susceptible" chromosomes. "If you take a pair of chromosomes and it doesn't have an exchange, it is in bad shape," explains Hassold. But here's the catch: Recombination occurs in the fetal oocyte before MI arrest sets in, so a 40-year-old woman has the same percentage of oocytes with susceptible chromosomes as she did when she was 20.

What does change with time, however, is the ability of the molecular players in meiosis to carry out their assigned roles. This is hit number two. Younger eggs, in other words, have a strong, healthy cast of stagehands, allowing these oocytes to disjoin even funky chromosomes. But as eggs age, parts of the cast—the spindle, for example, which helps pull chromosomes apart—begin to stumble, making nondisjunction of susceptible chromosomes increasingly common.



Split decisions. When meiosis works properly (left), pairs of homologous chromosomes divide evenly into four eggs. Errors in meiosis (right) lead to "aneuploid" eggs that have an extra or missing chromosome.

Divided minds

The two-hit model has won over many researchers, but some still have their doubts. Columbia's Warburton says she thinks the model is "a pretty good attempt" to explain how aberrant chiasmata and the maternal age effect fit together. "But it doesn't seem to fit all chromosomes," she cautions. Chromosome 18 in particular challenges the two-hit hypothesis. Many researchers have shown that trisomy 18 most often originates in MII, and in those cases, there's no evidence of reduced recombination in MI, indicating that a different mechanism must be responsible.

A model proposed by Roslyn Angell, who recently retired from the University of Edinburgh, U.K., purports to accommodate the findings in all chromosomes. Angell examined more than 200 oocytes rejected for IVF—clinicians routinely remove more eggs than needed and then select those they think are of the highest quality—and discovered a unique mechanism for aneuploidy.

Chromosomes are typically depicted with an X-shape because they consist of two sister "chromatids" that are Siamese twins of a sort. After fertilization, MII normally severs this bond to form single chromatids, with one set turning into the fe-

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male's contribution to the embryo and the other being cast off as trash. In Angell's studies, aneuploidy—without exception—resulted from sister chromatids prematurely separating in MI rather than MII, which ultimately can leave an egg shortchanged or overstocked with that chromosome. This precocious separation, Angell found, also occurs more frequently in older women, which leads her to suggest that the bonds between sisters become weaker over time. In her studies, this is particularly true for chromosome 16, the most common trisomy in humans.

Although some researchers questioned whether Angell's results were an IVF artifact, Case Western's Hunt and colleagues found evidence that premature separation occurs in a more natural setting, too. They studied 117 oocytes obtained during tubal ligations and routine gynecological surgeries and confirmed that premature separation occurs. But they doubt Angell's assertion that it explains all nondisjunction, because they also found whole chromosomes—not just chromatids—that did not properly separate.

A few theories about why humans suffer such high rates of aneuploidy build on much simpler notions of biology. One suggests that the longer the interval between sperm meeting ovulated egg, the more likely are abnormal embryos. Experiments have shown this is clearly the case in frogs, rodents, and other species, and it has led some to suggest that humans are so prone to aneuploidy because we do not have a mechanism, such as estrus, that optimizes the timing of fertilization.

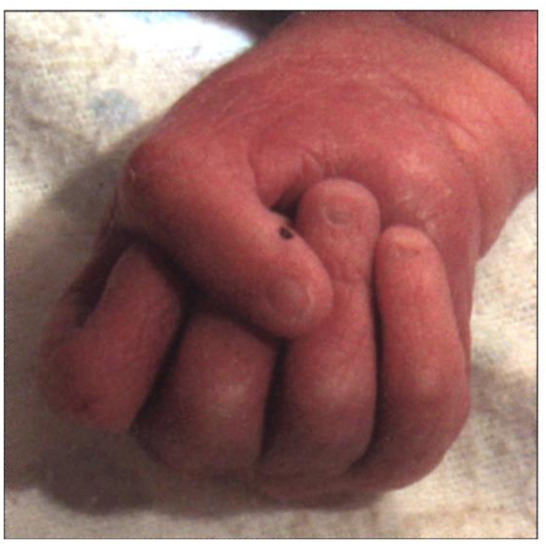
There's some evidence that mistimed meetings lead to aneuploidy in humans. In the NIEHS pregnancy study, the researchers found that women who became pregnant from intercourse that had occurred in the 5 days before they ovulated—and thus had viable sperm immediately ready to greet the egg—had significantly fewer miscarriages. A paper in the 11 May issue of *The Lancet*, however, finds no such association in a study of nearly 1000 women using natural family planning.

Columbia's Warburton has long sought evidence to support what she calls the "limited oocyte pool" hypothesis. Unlike the production-line idea, this scenario contends that it's not a question of the first eggs made in the fetus being the healthiest and the first ones to ovulate. Rather, it holds that aneuploidy occurs more frequently as women age because, as their stock of oocytes becomes depleted with time, fewer oocytes compete to become the one that is ovulated. This leads to more substandard oocytes making their way into the fallopian tubes. Warburton herself has reservations about the hypothesis. "As I've thought about it more, it's [become] unclear to me whether the pool is the cause of the problem or a sign of the problem," she says.

Spindle doctors?

Case Western's Hassold, who used to do genetic counseling with couples, says he'd love to figure out some way to help clean up messes made by meiosis. "My Holy Grail is to deliver some sort of aneuploidy-reduction pill," says Hassold. "The difficulty is there are a number of routes to aneuploidy."

Molecular studies might reveal the best places to intervene. The spindle, for example, appeared to deteriorate with age in both Hunt's study of oocytes and another that looked directly at non-IVF eggs. Using mutant mice, Hunt and colleagues report in the May issue of *Human Reproduction* that the spindle itself often appears abnormal when nondisjunction occurs. They suggest that aberrant hormone levels, often found in women as they approach menopause, might disturb the final maturation of oocytes, which in turn could affect the production of motor proteins that move chromosomes along the spindle. Recent studies have identified a bevy of other proteins that control many steps of meiosis, from the pairing of homologous chromosomes to the repair of mismatched DNA during recombination to the "cohesins" that glue sister chromatids together. Theoretically, treatments directed at one of these proteins might tune up meiotic engines and prevent them from stalling as frequently as they do.



One too many. People with Edward's syndrome, or trisomy 18, are born with distinctively clenched fingers.

A few aneuploidy interventions have already entered the clinic, although the results to date remain sketchy. One is called ooplasmic transfer, which builds on work that fingers defective mitochondria in the cytoplasm of eggs (the ooplasm) as a key suspect in aneuploidy. The theory suggests that as women age, these mitochondria become compromised and cannot produce as much adenosine triphosphate; that deficit prevents proper disjunction. To steer around this potential problem, Jason Barritt, Jacques Cohen, and co-workers at the Institute for Reproductive Medicine and Science of Saint Barnabas in Livingston, New Jersey, pioneered a technique to transfer ooplasm from a young woman's egg into the egg of an older woman.

In July 2001, the U.S. Food and Drug Administration decided that this procedure was a form of gene therapy and temporarily halted its use while reviewing the technique for approval. More than two dozen babies have been born as a result of this controversial procedure, which has been strongly criticized because it transfers the donor's mitochondrial DNA to the baby, creating a child with three genetic parents.

A less controversial but still unproven method of preventing aneuploidy that has made great headway in the past few years is preimplantation genetic diagnosis (PGD). In this IVF technique, embryos are screened for chromosomal abnormalities before they are implanted. The idea is that by reducing the number of aneuploid embryos, the IVF success rate should increase.

But geneticist Wendy Robinson, who studies aneuploidy at the University of British Columbia in Vancouver, Canada, cautions that PGD has several limitations. Given that IVF embryos often contain mosaic combinations of normal and aneuploid cells, PGD, which samples only one embryonic cell and typically only looks at a handful of chromosomes, might miss a lot of abnormalities, says Robinson. On the flip side, she notes, PGD might detect a mosaic cell that was destined to become the placenta, not the fetus. "I'd worry that you may be throwing away perfectly good embryos," she says.

Then again, Robinson, who works closely with a clinic in Vancouver that specializes in treating miscarriages, strongly supports the push by researchers to try to intervene. "When I was younger, I may have said 'It's nature's way,' " says Robinson. But, for career reasons, she did not have a child until she was 36. "It really has changed my views." And Robinson is confident that researchers will find ways to prevent many aneuploidies. "Eventually," she predicts, "we'll have more women having babies at age 40 than now."

—JON COHEN

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