the foreskin, a fifth finger, irregularity of toe length, etc. An enumeration of everything that could possibly be slightly off of perfect could scare any couple into not wanting to try to have children at all, but this is part of life. In any event, there clearly was no difference noted in this huge series of almost six thousand infants between the health of offspring of fertile couples and the offspring of ICSI and IVF procedures. For couples who wish more detail, I recommend they look up the scientific paper, which is located in the journal *Human Reproduction,* 2002, volume 17, issue no. 3, pages 671 to 694. However, I can summarize by saying that there is no greater risk of congenital abnormalities or other illnesses in children born via IVF or ICSI compared to those born via natural conception other than those related to high-order multiple pregnancy.

However, what still concerned people was that there might be some subtle intellectual or chromosomal genetic deficit of their babies not readily apparent at birth without careful genetic study. There is no difference in developmental rates or intelligence of any of these children from a normal, naturally conceived population. However, the risk of chromosomal errors that might not be immediately recognized on physical exam remained to be studied.

Understanding Genes and Chromosomes

It is critically important for all women having children at a later age to understand potential genetic or chromosomal errors that may not be readily apparent as congenital birth defects. Remember, we have said that when a woman is scraping the bottom of her ovarian pool of eggs, there will be a larger percentage of eggs that have chromosomal errors. This not only prevents pregnancy, but also increases the risk of Down syndrome or recurrent miscarriage. A similar phenomenon might occur with sperm from men who have extremely low sperm production, and thus might ICSI offspring have a similarly increased risk of chromosomal abnormality? To fully explore this, you first need to have a simple lesson in genes and chromosomes.

Genes are composed of long chains of DNA. The function of DNA is to direct by code the production of proteins. The amount and type of proteins that are produced under the direction of DNA determine your entire body structure and chemistry. That is how genes work. Your body,

and the body of every animal on this planet, is made up of a variety of proteins, and these proteins are constructed from just twenty amino acids. There is an almost unlimited variety of amino acids that can be synthesized by chemists. However, in nature, on planet Earth, everything that is alive is composed of just twenty of those many hundreds of possible amino acids. This set of twenty amino acids is absolutely the same for all living beings on Earth, whether a fruit fly, an elephant, or a human. The reason that all of us are so different simply lies in the different ways that these amino acids are sequenced, which determines the characteristic protein structures of that particular being. The chemical structure of DNA is also the same for all animals on the earth. There is no difference between the chemistry of DNA in you or in a worm. In that sense, we are all the same.

In fact, DNA is nothing more than a long polymer, a molecule that just goes on and on in one long chain. Plastic is such a chain molecule. DNA (like plastic) is also a chain molecule consisting of four main ingredients: adenosine (A), thymidine (T), guanine (G), and cytosine (C). In scientific journals these ingredients are routinely referred to simply as the four letters A, T, G, and C.

The sequence in which these four basic "letters" occur in a vast sequential array on the DNA molecule represents a code that instructs the cell exactly which way to sequence its amino acids so as to construct each particular protein. This DNA code was first deciphered in 1967. Each of the twenty amino acids that make up a protein is matched by a very specific, definite codon, a sequence of three of the four DNA letters arranged in a specific order. The specific code whereby DNA instructs the sequence of amino acids to construct proteins is universal in all animals on Earth, and is no different in you and me from the toad, the guinea pig, or even a lowly bacteria.

There are 64 possible different ways in which these four letters (A, T, G, C) can be arranged, grouped three at a time, but only twenty amino acids they need to encode. Therefore, several different three-letter combinations can each encode the same amino acid. However, not all of these sixty-four codons produce a specific amino acid. There are several "nonsense" codons that serve as stop signs or start signs, telling the gene at what point to begin laying down the amino acids to create a protein, and at what point to stop. Thus, most amino acids are matched by several possible codons, and there are a few codons that have no amino acid match.

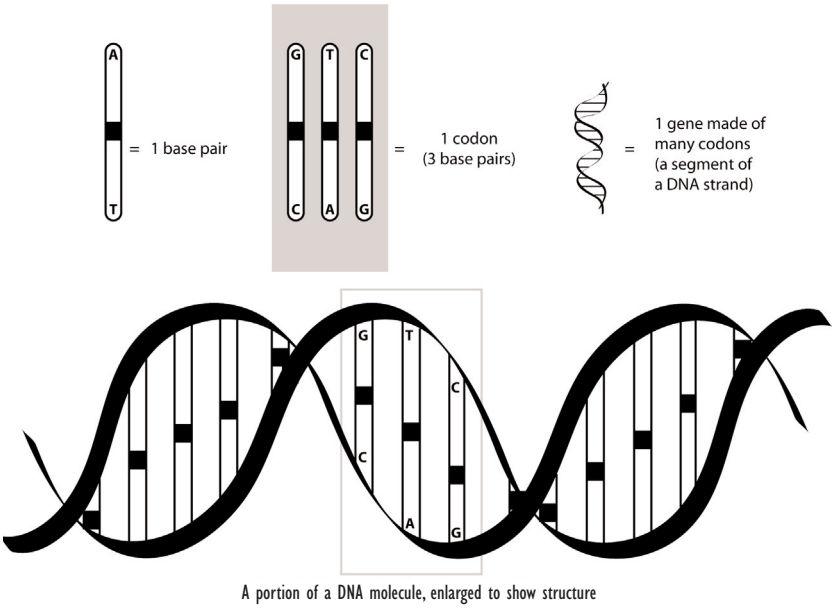

FIGURE 12.5. The relationship between genes and DNA.

These letters of DNA, which encode for amino acid sequence and, therefore, protein structures, are often referred to as nucleotides, or base pairs. Either way, in most scientific journals, they are simply referred to as the letters A, T, G, and C. It is the sequence of these three billion letters of A, T, G, and C from our father's sperm and our mother's egg that determine, at least structurally, who we are. Each molecule of DNA actually comprises two separate chains of letters wrapped around each other in a double helix, much like a spiral staircase (see fig. 12.5). The nucleotides, i.e., A, T, G, and C, comprise the coding side of this double helix. The other side is just a generic sugar molecule. A single length of DNA is composed of two sets of base pairs that are intimately attached and identical to each other. The A of one strand is always attached to the T of the other strand, and the G of one strand is always attached to the C of the other strand. A base pair is simply an A lined up with a T, or a G lined up with a C. Wherever there is a T, its opposite mate is an A, and wherever there is a G, its opposite mate is a C. This complementary system allows DNA strands to duplicate themselves exactly, the one strand serving as a template for the creation of another identical strand. It is this organization that allows a chromosome to divide into absolutely identical sub-

units during cell division so that every single cell of the ten trillion cells in our body is genetically, if not functionally, identical.

A chromosome is simply a huge, long chain of literally millions of base pairs of the A, T, G, and C components of DNA. Understanding your chromosomes will allow you to understand better how your fertility clock works and will help you decipher the otherwise confusing array of "scare" articles you may have read regarding whether to expect a healthy, normal baby.

Base pairs in a single strand of DNA merely represent one chromosome, and every cell in your body (except for sperm or mature eggs) has two sets of chromosomes. Each set of three base pairs represents a code and, therefore, is called a codon; thousands of these codons make up a gene. There are approximately a thousand or more genes in every one of your chromosomes, and each one of these genes consists of many thousands of DNA base pairs lined up in a specific sequence. Thus, all of life on Earth, no matter how complex and varying, is made up of these same four base pairs, which code in the same exact way for the same exact twenty amino acids to produce a variety of different proteins that account for the incredible variety of life.

Most of the cell's DNA is enclosed within the nucleus of the cell. The nucleus is about twenty microns in size, which is thinner than the diameter of a single hair. All of the genetic information that codes for what you are is crammed into this little package. A total of six billion base pairs, three billion for each set of chromosomes, are located in that tiny twenty-micron nucleus located within each cell. To gain an understanding of this remarkable feat of nature, just imagine this: If the chromosome were actually three millimeters thick (approximately 1/15 of an inch), then all of the chromosomes within a single cell would stretch all the way from the East Coast of the United States to the West Coast.

Each cell in your body has forty-six such chromosomes (two pairs of twenty-three) (see fig. 12.6). Forty-four of them are called autosomes because they have nothing to do with sex determination. The other two are the X and Y chromosomes. The chromosomes are arbitrarily numbered and designated in order of decreasing length. Thus, the largest human chromosome is chromosome 1, and the smallest human chromosome is chromosome 22. The human X and Y chromosomes are separately named because they are inextricably connected to each other throughout evolutionary time and must work together. The X chromosome's

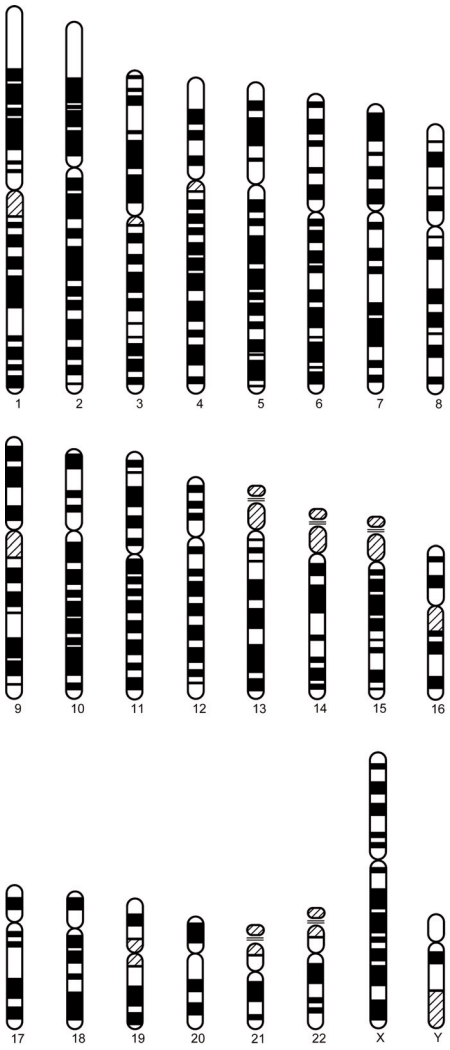

FIGURE 12.6

A diagram of all twenty-two human autosomal chromosomes, as well as X and Y. The dark-staining regions are densely repetitive DNA, and the light-staining regions are nonrepetitive sequences. These staining patterns are used to identify the chromosomes.

size falls between that of chromosome 7 and chromosome 8. The Y chromosome is very tiny, and it is the chromosome that determines whether the embryo will become a boy or a girl. It also harbors many genes specifically designed for proper sperm production.

An Abnormal Number of Chromosomes Causes Down Syndrome and Miscarriage

Two copies of each chromosome are absolutely necessary for the embryo to develop into a normal fetus and a healthy baby. If there is an extra copy of one of these chromosomes, or a missing copy of one of these chromosomes, leading to perhaps forty-seven chromosomes or forty-five chromosomes in the fertilized egg, the embryo cannot possibly develop normally. That is why fetuses with three copies of chromosome 21, instead of two, either miscarry (80 percent of the time) or result in a Down syndrome baby. In fact, the reason that Down syndrome (trisomy 21) is so feared is that it is one of the few chromosomal abnormalities that is not programmed for complete death and miscarriage, but rather can result in an occasional, but abnormal, live offspring. A glance back at figure 12.6 will explain the reason that individuals with three copies of chromosome 21 can survive. Chromosome 21 is probably one of the smallest chromosomes, and has the fewest genes. Thus, an overdose of genes from an extra chromosome 21 would not be as likely to be lethal as an overdose of any of the other autosomes. Trisomy means three copies rather than two copies of a particular chromosome. A trisomy of chromosome 1 would not be compatible with embryo development because chromosome 1 is huge, and it harbors many genes critical for life function. You wouldn't even see errors in chromosome 1 in a fetal miscarriage because the fertilized egg simply would not develop far enough even to implant.

However, when chromosomes 13 and 18 are present in three copies instead of two, although ultimately lethal, this can occasionally result in advanced fetal development and even a stillbirth. In fact, any of the chromosomes larger than chromosome 12, if present in three copies instead of two, can result in pregnancy but will ultimately end in an early miscarriage. The reason for this miscarriage is not some defect in the mother's uterus or her inability to "hold the pregnancy." Rather, it is simply because the embryo was programmed for death from the very beginning. In contrast to trisomy (three copies of a chromosome) when there is only one copy of a particular chromosome (monosomy), this is almost always fatal, and with the exception of the sex chromosomes, it is unlikely to lead even to a hint of early pregnancy.

How Your Aging Eggs Develop Chromosome Errors

How do these chromosomal errors occur? How does this problem of too many or too few copies of a chromosome occur in at least 15 percent of pregnancies even in young, fertile women, and in well over 60 percent of the pregnancies of older women? Actually, the majority of human embryos are chromosomally abnormal, and most of these abnormalities are present in the fertilized egg in the first few hours of life. These common chromosomal errors (where you have either one too many or one too few of a particular chromosome, or of several chromosomes) in 90 percent of cases are a result of how the egg prepares for fertilization by reducing its chromosome number from forty-six to twenty-three. In 10 percent of the cases, it is a consequence of how the sperm is produced in the testes (also when sperm precursor cells reduce their number from forty-six to twenty-three). This process of reduction from forty-six to twenty-three chromosomes occurs only in the egg or in the sperm (no other tissue in the body) as they are preparing for fertilization. This process is called meiosis. Most chromosomal errors that would result in a Down syndrome child, a miscarriage, or simply a failure to get pregnant arise from faulty meiosis.

I will now explain exactly how meiosis occurs and try to simplify this very complex process so that you can understand just how the chromosomes can get accidentally mis-shuffled, and what we can do about it. But it is a little tricky to understand, and those who aren't interested may wish to skip to the next subsection.

Take a look at figures 12.7 and 12.8, which show how most cells in your body divide and replenish themselves. During most of the cell's life, the chromosomes are very loosely entwined with one another in the nucleus and are working at directing the production of the various proteins, which take care of the cell's growth and metabolism. Your chromosomes, most of the time, look like a curly, jumbled mass of wires, similar to a Brillo pad, inside the cell's nucleus. However, when it comes time for the cell to divide, the chromosomes line up in an orderly way on a special structure called the spindle. When the cell divides, each chromosome duplicates, and one set of chromosomes goes on its way along the spindle to one cell, as the other set of chromosomes goes on its way to the other cell. Every cell in the body needs to do this in order to divide. This process of identical cell division, with exact duplication

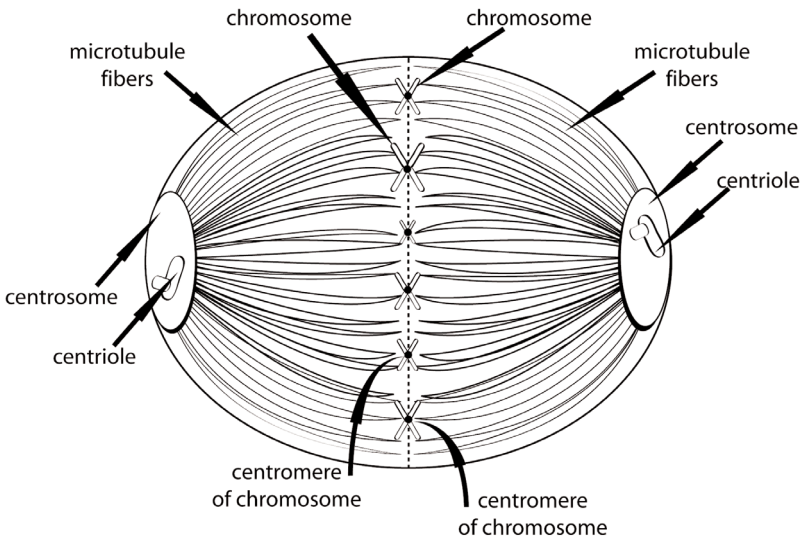

FIGURE 12.7. Mitotic cell division: chromosomes lining up on spindle prior to separation.

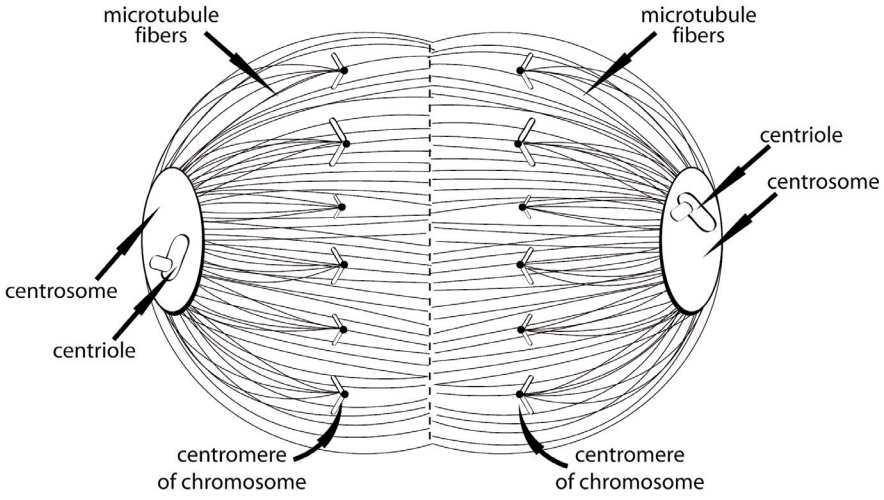

FIGURE 12.8

Mitotic cell division: spindle motor network for separating chromosomes at the time of cell division; chromosomes are now receding toward opposite poles along this spindle.

(Xeroxing) of the chromosomes for the next generation of cells is called mitosis. This is the process whereby every cell in the body (except for sperm and eggs) duplicates itself.

The cell, in preparation for ordinary division, assembles this "scaffold," the spindle, upon which all chromosome movement occurs. The lines of the spindle are like train tracks along which the centromere (the central holding structure of the chromosome) can find its way rapidly to opposite poles of the dividing cell. The centromere is the motor, which pulls the chromosome along the spindle, and the spindle is what guides the chromosomes to opposite sides.

It is this cell division, with exact replication of genetic material, that allows entire individuals to develop from an initial single cell into a complex human being of approximately ten trillion cells. The only difference between the chromosomes in any of our cells (remember, they are genetically identical) is that certain genes are "turned on" and certain genes are "turned off" depending on which tissue or body part they reside in. The genetic blueprint is the same in every cell in our body, and what makes the cells function differently in different parts of our body (with the exception of general housekeeping genes) is only that some genes are turned off, and others are turned on, but they are all present.

The sperm and the egg undergo a much more complex process called meiosis. During meiosis problems can arise with the aging egg, which has been sitting around for many decades (in forty-year-old women, for four decades) waiting for this moment. The aging of the egg causes the spindle apparatus to become so dysfunctional that chromosome separation gets mishandled.

Meiosis involves two different processes of cell division. In the first phase, very much like mitosis, each of the two copies of every chromosome pair begins to divide and is connected only by the centromere, that common, central glue point that holds the chromosomes together (see fig. 12.9). These two sets of chromosome pairs line up next to each other on the central plate of the spindle, but do not actually complete their division yet. Their duplication does not result in complete separation because the centromere holds fast and does not, itself, separate. These homologous chromosomes then exchange genetic material (in a process called recombination). Without dividing, they get pulled away from each other along the tracks of the spindle as their centromeres move to opposite poles. This is the so-called first meiotic division.

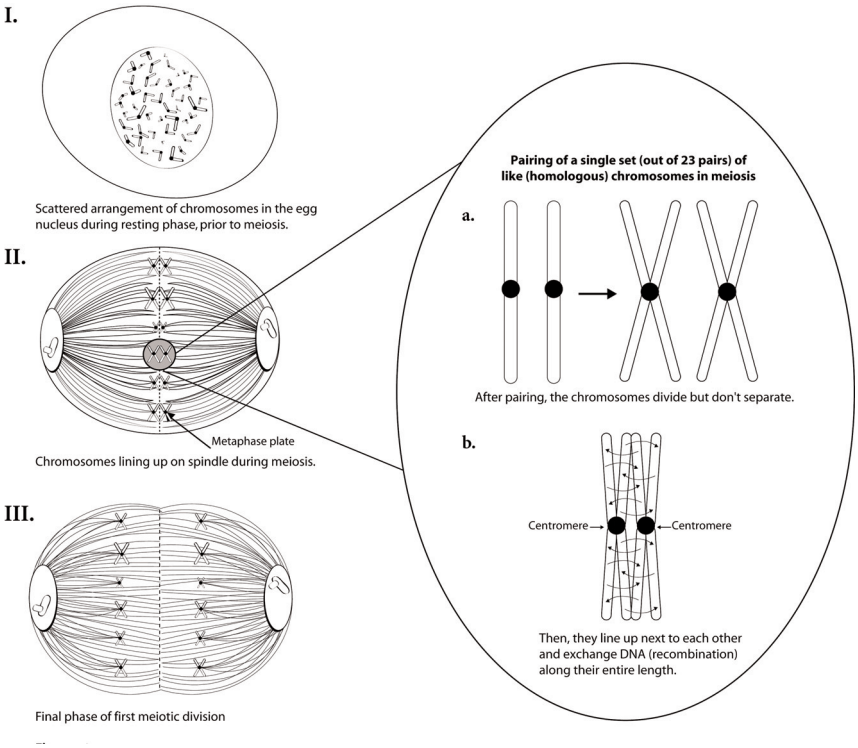

FIGURE 12.9. First meiotic division.

In the second meiotic division, the chromosomes that had originally divided but were still held together by their centromere finally finish their division as their centromeres divide. It is during the shuffle of these two phases of meiotic division, when chromosomes first become duplicated, then separate, and then divide later, that an error can occur in the number of chromosomes that wind up in the egg or the sperm. If the spindle is not working properly, because of an aging process inherent in the egg that becomes more and more exaggerated as the woman gets older, there may either be an extra copy of one or several of the chromosomes in the egg, or there may be a copy missing of one or several of the chromosomes (see fig. 12.10). That is the cause of increasing infertility with the woman's age, miscarriage, and chromosomal birth defects such as Down syndrome (see fig. 12.11).

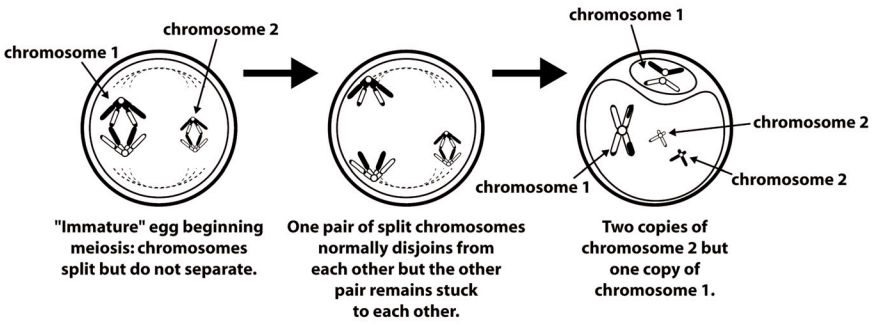

FIGURE 12.10

Development of aneuploidy in eggs: failure to separate on the meiotic spindle.

Is It the Egg or the Sperm That Is at Fault?

In the male, new sperm production is continually going on every day. Meiosis for sperm, as opposed to eggs, begins with spermatogenic stem cells, and is a continuing, renewing process. The female's eggs first begin the process of meiosis when she is just a fetus; the eggs remain perpetually frozen in that state of early meiosis until they eventually resume meiosis when they ovulate. In males, the meiotic process, by contrast, is fresh and free-running, with cells progressing daily through meiosis in an uninterrupted fashion. In females, the long delay over many decades in completing the meiotic division is the reason for the dramatic increase in birth defects seen in older women. Ninety percent of chromosomal errors in human embryos come from the egg while only 10 percent come from the sperm.

There is, however, another important difference, specifically in humans, between male meiosis and female meiosis. The neck of the sperm has a round structure on it that joins the sperm head with the sperm tail. This is called the sperm centrosome. Every cell in your body must have a centrosome in order to pull the centromeres of the divided chromosomes to the opposite poles of what will become two new cells when it divides. Whether you're a male or a female, the centrosome of every cell in your body was inherited from your father's sperm. In the egg of the human female, however, there is no centrosome. In order to perform meiosis, the egg's chromosomes must bind themselves to the spindle, which has to be built from inside out without any assistance from a centrosome. After fertilization, the sperm centrosome (once it

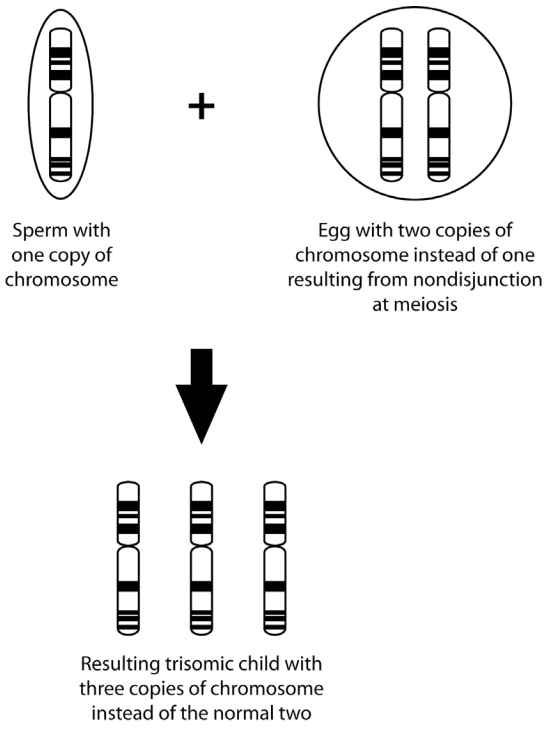

FIGURE 12.11

Diagram showing how three copies of a chromosome (instead of the normal two) can find their way to what becomes the trisomic Down's infant because of nondisjunction in the egg's meiosis, with two copies (instead of one) in the original egg.

enters the egg) divides and becomes the centrosome of the egg, which is necessary for all future development of that fertilized egg into a normal embryo, and subsequently into a baby.

The centrosome of the sperm is thus crucial for development of the fertilized egg through all of its embryologic and fetal stages. The centrosome remains necessary for the maintenance of all adult life. Cell division is the basis of all life, and it cannot occur without the proper, equal separation of chromosomes, which occurs because of this chromosomal motor, which comes from the sperm. Therefore, the entrance of an intact, normal sperm is essential for human embryo development.

Nonetheless, in more than 90 percent of cases, the cause of chromosomal errors in the embryo is the aging egg. The aging egg is the origin of the woman's biological clock. It is the cause of her infertility, her

miscarriages, and her increasing risk of having an abnormal child as she gets older.

Chromosomal Errors (Aneuploidy) Versus Single-Gene Defects

There are two types of genetic errors in newborns — chromosomal errors and single-gene defects. The term that is commonly used for chromosomal errors is aneuploidy. Aneuploidy simply means that there are either more than two or less than two copies of one of the chromosomes. Down syndrome is caused by an aneuploidy of chromosome 21, often called trisomy 21 because there are three copies of chromosome 21 rather than the normal two copies. Aneuploidy of any of the 22 autosomes is either lethal or causes severe abnormalities incompatible with life (as in trisomy 13, 18, and 21).

All chromosomal abnormalities in a fetus can be diagnosed by either CVS (chorionic villus sampling) or amniocentesis during early pregnancy. CVS can be safely performed as early as ten or eleven weeks of fetal life, and amniocentesis can be performed as early as fourteen weeks of fetal life. With CVS, a tiny piece of the developing placenta of the embryo is aspirated and classically the cells are cultured for several weeks so that the chromosomes can be stained and observed. However, a quick answer can be obtained by the next day using a direct staining process called FISH (which I will explain fully in the next chapter). Amniocentesis is very similar to CVS except that the needle is placed directly into the amniotic cavity of a somewhat more developed pregnancy. Amniocentesis is much more popular than CVS because it is easier and requires less-specialized training, though both are successful methods for diagnosing virtually any abnormality resulting from a chromosomal error. However, CVS will give you an answer much earlier in pregnancy.

Most aneuploidies result in an early miscarriage before CVS or amniocentesis can even be attempted. However, trisomies of chromosome 13, 18, and 21 will often survive far into pregnancy and can occasionally lead to birth. It is mostly for that reason that CVS or amniocentesis is advised for women over the age of thirty-five. Congenital anomalies not related to chromosome error are generally only diagnosable by ultrasound, and often at a later stage in the pregnancy (typically at eighteen

weeks). The major reason most women undergo CVS or amniocentesis is to detect Down syndrome.

Chromosome analysis (karyotyping) will not pick up subtle gene defects that are often referred to as single-gene errors. Karyotyping only detects gross errors such as an abnormal number of chromosomes or a structural defect in a chromosome. Remember, there are about twenty-five thousand genes located in these twenty-three pairs of chromosomes. Each gene (except on the X) has two copies and consists of a sequence of many thousands of DNA base pairs. A mistake in just a single one of those base pair letters can result in a severe disease such as cystic fibrosis or muscular dystrophy. This will not show up on routine chromosome analysis because the DNA defect is tiny compared to a missing or extra chromosome. There are many, many single-gene defects, some of which you may have heard of, such as cystic fibrosis, Tay-Sachs disease, muscular dystrophy, sickle cell anemia, Gaucher's disease, Marfan syndrome, and hemophilia, just to mention a few. But unlike aneuploidy, they are very uncommon. These are diseases caused by specific defects in a tiny area of a gene that would go completely unnoticed with any karyotype analysis (i.e., chromosomal examination). They are caused by mutations rather than numerical chromosomal errors.

With aneuploidy, there is a numerical error in the number of chromosomes present, representing a huge chunk of DNA (possibly one hundred million base pairs) that is clearly visible upon microscopic examination. On the other hand, a mutation represents a tiny, submicroscopic defect in the organization of DNA letters within a specific gene. The most common single-gene defect is cystic fibrosis, which occurs in about 1 in 1,600 offspring. The remarkable aspect of these single-gene defects is that most of us are carriers for at least ten such fatal diseases. In fact, 4 percent of the Caucasian population are carriers of cystic fibrosis, even though only 1 in 1,600 children actually have the disease.

These single-gene diseases are nonetheless uncommon for two reasons. First, if the genes are recessive, the child must inherit the mutation from each parent, both of whom must be carriers. If just one healthy gene from one of the parents is present, then the child will be disease-free. Cystic fibrosis functions in this way. Thus, although 4 percent of the entire population are carriers for this disease, the chance that any

two people who are both carriers will marry is 4 percent of 4 percent. That means that only sixteen out of ten thousand babies would be born to couples in which both mother and father are carriers. Furthermore, only one quarter of those babies would receive a defective recessive gene from both the father and the mother. Three quarters of those babies would either have no defective genes, or be carriers themselves. Thus, only about 1 in 1,600 children are actually born with this terrible disease, even though 4 percent of the population are carriers for it. There is a huge variety of these mutations, but only if we are very unfortunate, and happen to have married a similar carrier, is there a danger we could have an offspring affected by the disease.

On the other hand, there are also single-gene diseases caused by so-called dominant genes, which means that if just one, not necessarily two, of the genes you inherit from your father or mother is abnormal, you will have the disease. Classic examples of these are Huntington's, Marfan's, myotonic dystrophy, and polycystic kidney disease. These are terrible diseases that affect people who have just one abnormal gene out of the two. Even though the homologous gene is normal, they still have the disease. This means that if your father had Huntington's disease, there is a 50 percent chance that you will have it as well, depending randomly on whether you received your father's defective Huntington gene or his normal Huntington gene. Autosomal dominant disease, therefore, is usually only an adult-onset condition that occurs after the early childbearing years, and is genetically transmitted to half of the offspring.

Chromosomal Translocations and Infertility

Aneuploidies, or numerical chromosome errors, as we have discussed, result from having either more or less than the normal two copies of any one of the chromosomes. Aneuploidy is caused by errors in meiosis in either the egg (90 percent of the time) or the sperm (10 percent of the time). However, aneuploidy can also occur from mis-segregation of chromosomes caused by a phenomenon referred to as translocation. This means that a portion of one chromosome is "translocated" onto another chromosome. Translocation is actually a normal part of evolutionary development in the creation of new species. About one in every four hundred adult men and women have such a transloca-

tion in their chromosomes, and they are completely normal individuals, suffering no genetic or health consequences. A translocation simply means that part of one chromosome has been broken off and moved to another. As long as there is no addition or subtraction of genes caused by this translocation, it doesn't really matter where the genes are located. As long as the nucleus of every one of your cells has the normal number of genes, even if the chromosomes have been split apart and rearranged like a randomly shuffled deck of cards, translocation has no negative consequence.

With reciprocal translocations, there is simply an exchange of chromosome material from one non-homologous chromosome to another. Thus, a chunk of your chromosome 13 might be located on chromosome 14, and a chunk of your chromosome 14 might be located reciprocally on chromosome 13. This has no negative effect on your health as long as there is no missing or extra chromosomal segment. In another type of translocation, called Robertsonian translocation, the centromeres of two chromosomes are fused, and so one entire chromosome is thereby completely attached to another entire chromosome. This type of translocation is only found with chromosomes 13, 14, 15, 21, and 22. Since there is no health consequence to you if you carry a Robertsonian or a reciprocal translocation, these chromosome errors are referred to as balanced, and these individuals are completely healthy.

Translocations occur throughout evolution, as large or small chunks of chromosomes can dislodge and become attached to other chromosomes. In fact, this is one of the major mechanisms whereby species differentiate, and the reason that you can't cross one species with another. The chromosomes simply would not match up. Occasionally, cross-breeding between closely related species is possible. For example, the crossing of a horse and a donkey yields a mule. However, because the chromosomal organization is essentially different in the donkey and the horse, the mule is sterile and cannot breed further mules.

So why are these translocations important to you and your desire to have a child? The problem is that at the time of either the production of sperm or the maturing of the egg via meiosis, an equal separation of chromosomes must occur, with one pair of chromosomes going to one pole of the dividing egg (or sperm), and the other equivalent set of chromosomes going to the other pole. If you have a balanced translocation, the chromosomes cannot separate off equally in meiosis

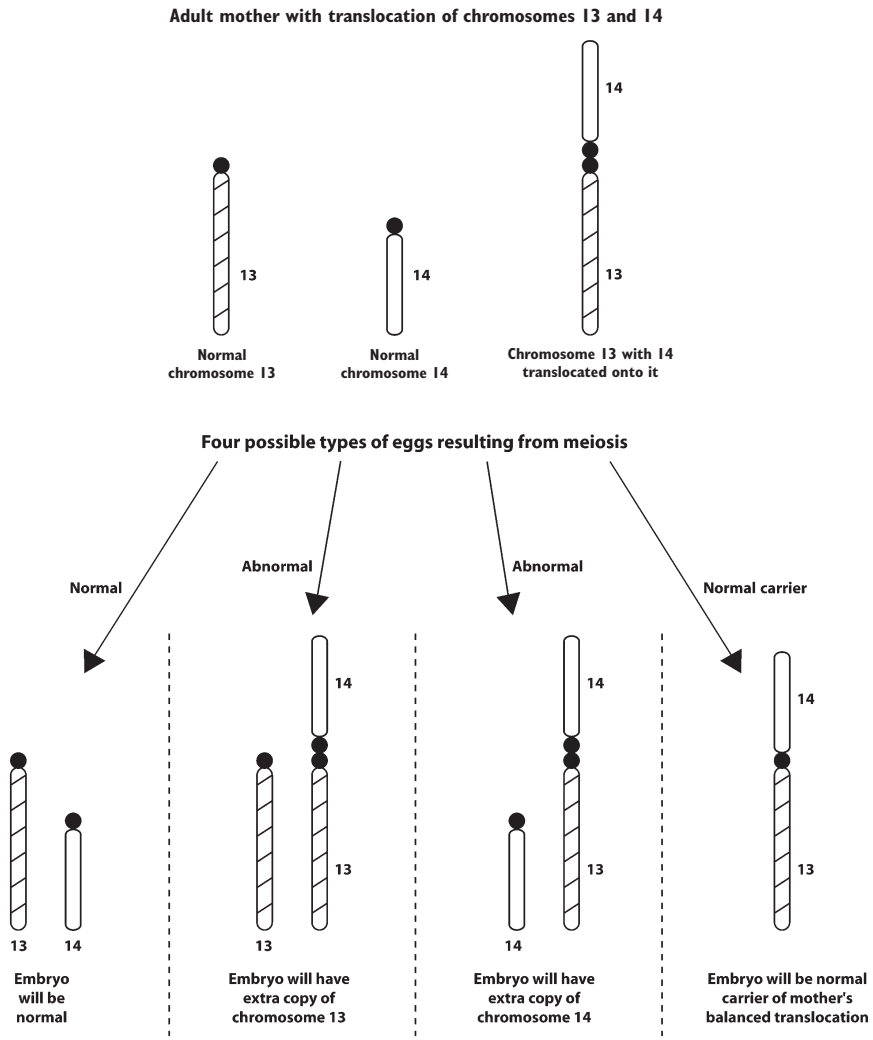

FIGURE 12.12

How balanced translocations in 1.3 percent of infertile parents lead to normal and abnormal embryos.

(see fig. 12.12). Thus, if chromosomes 13 and 14 are fused together (Robertsonian), there will be eggs that have the fused pair of both 13 and 14 as well as another extra copy of one of those chromosomes. In other words, the chromosomes have to be intact and paired so that an equal amount of chromosomal material goes to each sperm, or to each egg, during meiosis.

The simple math (although it doesn't always work out this way) would be that one quarter of the offspring would have inherited the balanced translocation from its parent and be no different from that parent. One quarter will inherit no translocation and will just inherit the normal chromosome of each one of the parents. However, half of the embryos resulting from a parent with a balanced translocation will have an unbalanced number of chromosomes (see fig. 12.12). The abnormal meiotic separation of chromosomes in the presence of translocations produces aneuploid gametes not because there is an extra or a missing chromosome in the fetus, but rather because there is an extra or a missing *portion* of a chromosome. This results in miscarriages as well as abnormal offspring. Fortunately, translocations are only found in a small percentage of the general population (about 1 in 400). But they are found in as many as 1.5 percent of infertile men and women. The offspring of these patients have a 50 percent chance of having the same problem as their parents and at least half of their conceptions will be nonviable.

Sex-Chromosome Aneuploidies

Thus far, we have concentrated on explaining aneuploidy of the *autosomes.* Aneuploidy of the autosomes, i.e., chromosomes 1 through 22, leads to either a nonviable embryo that cannot implant at all, a viable embryo programmed to die sometime in the first three months (miscarriage), or, occasionally, a stillbirth or a child affected with Down syndrome (trisomy 21). However, aneuploidies of the sex chromosomes can be quite viable and may result in a real dilemma for every patient undergoing assisted reproductive technology with IVF or ICSI. ICSI offspring have a slightly increased incidence of sex-chromosome aneuploidy, so you need to understand it.

Sex-chromosome aneuploidy means that there is either an extra or a missing copy of the X or the Y chromosome. We know that two copies of the X chromosome makes a normal female, and that one copy of the X and one copy of the Y makes a normal male. The X and the Y chromosomes are very unusual compared to the autosomes. The X is rather large, between the size of chromosome 7 and chromosome 8, and contains more than one thousand genes. The Y chromosome is a very tiny, puny little mate by comparison, representing only 1 percent of the

entire human genome. The Y chromosome is approximately the same size as chromosomes 21 and 22, which are also very small, and it is dwarfed by its corresponding mate, the X chromosome.

For many years, it was thought that the only purpose of the Y chromosome was to provide the male sex-determining gene (SRY), because women with two X chromosomes certainly don't need the Y, and even women with a single X chromosome (XO Turner's syndrome patients) can get along (with some handicaps) without a Y chromosome. Nonetheless, the Y chromosome has important genes on it, as exemplified by the fact that 99 percent of XO aneuploid embryos miscarry within the first three months and are completely nonviable. It is only a tiny minority of embryos with only one X chromosome (no second X, and no Y chromosome) that survive. Therefore, the X chromosome is absolutely essential for survival, but interestingly, only one copy of it is necessary so long as there is a corresponding little Y chromosome to accompany it.

This can best be understood in terms of the whole evolutionary history of the X and the Y chromosomes that has occurred in mammals for hundreds of millions of years. The X and the Y chromosomes actually started out as a pair of ordinary autosomes approximately the size of chromosomes 7 or 8. Before the development of the Y chromosome, the gender of the offspring in our ancient reptilian ancestors would be determined simply by the randomness of the temperature at which the egg was incubated. We see this today, of course, in alligators, crocodiles, and many turtles. This sex-determining gene ensured a balanced, fifty-fifty sex ratio in spite of all kinds of unpredictable vicissitudes of environmental temperature, but it also guaranteed the gradual atrophy and shrinkage of what would eventually become the Y chromosome. Chromosomes that do not pair during meiosis gradually deteriorate. But the evolving organism cannot survive with only one chromosome of a given pair. Thus, the evolving X chromosome developed a way to work overtime to make up for the loss of genes from its mate, the Y.

The female X chromosome works twice as hard as any other chromosome, making up for the absence of the vast majority of the genes from its deteriorating mate, the Y. However, this hyperproduction of the X chromosome, though saving the life of the male by making up for the deficiency of the Y, would be disastrous for the female (with two copies

of X chromosome) were it not for another phenomenon called X inactivation. The female (XX) actually inactivates one of her X chromosomes so that she doesn't suffer from a gene overdosage. In this way, a normal XX female basically has only one of her X chromosomes functioning, and a normal XY male also has only one X chromosome functioning. This can help to explain why sex-chromosome aneuploid embryos can survive. You will have to decide whether they present enough of an abnormality for you to be concerned for your offspring.

The most common of these sex-chromosome aneuploidies is XXY, or Klinefelter's syndrome. The vast majority of men with sex chromosome trisomy, XXY, appear to be completely normal and healthy. In fact, they live completely normal lives and will go undiagnosed as having this sex-chromosome aneuploidy until they try to have children and discover that they have no sperm in their ejaculate. There are less common, more severe cases of XXY Klinefelter's whereby testicular function is so compromised that even hormone production is deficient, and such a patient would never go through normal puberty. He would need testosterone supplementation, usually via injection twice a month, in order to live a normal life. Furthermore, he would be sterile and unable to have his own genetic offspring. But the majority of XXY males are perfectly normal, except that they are infertile.

The earliest reported cases of Klinefelter's were not detected by routine genetic testing of an otherwise normal male coming in for an infertility evaluation. Therefore, only the more severe cases, in which there was obvious male hormone deficiency, were diagnosed in the early days. These men had all the signs of testosterone deficiency, including lack of drive, bone weakness, relative paucity of facial and pubic hair, deficient muscle mass, and a lack of proper pubertal development. That is why for many years, a controversy has raged about whether these XXY males have some sort of mild retardation. But we have seen more XXY men than perhaps any other center, and aside from their infertility, these men are 100 percent normal. Many are quite brilliant, no different than in any normal population of men.

Many years ago, I remember being shocked by a routine chromosome report showing Klinefelter's on a patient who was an extremely successful and highly regarded businessman and who never would have known he had an XXY aneuploidy were it not for the fact that he and his wife were trying to have a baby. Chromosome evaluations (karyotyping)

in the past had not been routinely performed for infertile men. But as we began to routinely screen all of our infertile male patients, we discovered that 4 percent of our men with azoospermia (no sperm in the ejaculate) actually had Klinefelter's. There was nothing else wrong with them.

Sex-chromosome aneuploidies, regardless of age of the parents, are found in only 0.2 percent of infants (1 in every 500) in a normal newborn population. However, they are found in 0.8 percent (1 in 125) of offspring from severely infertile males requiring ICSI. This is approximately the same as the incidence of Down syndrome found in infants of thirty-eight-year-old women. Therefore, much like every woman over thirty-five who has to decide whether to have amniocentesis to determine whether or not the pregnancy is normal, every couple undergoing ICSI for severe male infertility has to make a similar decision, depending upon how much concern they have over the less than 1 percent possibility of a sex-chromosome aneuploidy. However, sex chromosomal abnormalities related to ICSI are very minor events compared to the risk of Down Syndrome in older women. All of these infants will appear to be completely normal at birth. The sex-chromosome aneuploidy may never even be discovered unless, during an infertility evaluation later in life, the man undergoes chromosome testing.

There are numerous other sex-chromosome aneuploidies that are less common than XXY. XYY males are fertile, and almost all of them lead normal lives. Most of them never even find out that they are XYY. In fact, XYY occurs in about 1 in 1,000 babies born in a normal population. Some poorly contrived studies suggested that the frequency of XYY is somewhat higher in prison populations than in a normal population. The suggestion has been made that XYY males may tend to be more aggressive than XY males because of the increased "maleness" of having an extra Y. In fact, this supposition is complete myth.

XXY males, as we have said, will grow up to be infertile, and the most severe (but least common) variety of the XXY males will need testosterone replacement to lead normal lives. The majority, however, will not need testosterone replacement, and will simply be infertile. Whether or not these XXY males are completely normal except for infertility is a crucial issue for couples undergoing ICSI, since up to 0.8 percent of ICSI offspring will have such a sex-chromosome anomaly. This is a potentially controversial issue, but we have interviewed so many of these

XXY men whose only problem was azoospermia that we feel that XXY children are just as likely to be rocket scientists as any other population of males.

Other sex-chromosome aneuploidies include XXX and XO females. Even with ICSI, the XO sex-chromosome aneuploidy is very uncommon at birth because it is usually lethal to the early embryo, and results in early miscarriage. But it poses a problem to prospective parents if such a pregnancy actually were to result in the birth of an XO baby girl. XO (also called Turner's syndrome) is extremely rare (0.01 percent) because these fetuses simply do not survive 99 percent of the time and miscarry very early. But when they do go to birth, these girls will grow up to be very short (less than five feet tall), some will have a webbed neck, and some will have one horseshoe-shaped kidney instead of two normally shaped kidneys. It is also alleged that they do not have high intelligence, but that is very disputable.

The head professor and chief of the Department of Genetics at the University of Amsterdam, who has studied this phenomenon extensively, relates a story that is now well known among geneticists. There was a female first-year medical student who was quite brilliant, already at the top of her class, listening to a medical genetics lecture. The professor went through a list of birth disorders and explained XO Turner's syndrome. The professor explained, "The Turner's syndrome female carries only one X chromosome due to a loss of one of the X chromosomes during meiosis, or early embryonic mitosis. She may be of shorter stature, possess rudimentary ovaries, thus making her sterile, have immature breasts and general development, and usually will be of below-average intelligence." This medical student knew that she had XO Turner's, and could not believe that this professor, who was well respected and learned, would be making such incorrect statements, at least as it applied to her. Afterward, she spoke to the professor and explained to him that she was at the top of her class and that she was a Turner's syndrome XO female. The next day, the professor apologized and said, "There is nothing written in stone about how these people will live their lives and what their mental capacity will be." This is a very famous anecdote, and it emphasizes that despite fears couples might have about discovering sex-chromosome errors in their offspring, either at amniocentesis or in early childhood, it is unlikely that many of these uncommon sex-chromosome aneuploidies will lead to any heartbreaking problems other than infertility.

Severely Infertile Males and Sex-Chromosome Aneuploidy

Sex-chromosome abnormalities come from the sperm rather than the egg, and are not increased in older parents. They are *not* the result of the biological clock of the eggs, but rather of errors in meiosis of sperm.

It is the Y chromosome that determines, at approximately six weeks of fetal life, that the embryo will become a male. Even Y chromosomes that are missing large chunks (a type of chromosomal error called a deletion) that are easily visible with karyotyping, still are males. There is just one tiny region (the SRY gene) on what is called the "short arm" of the Y chromosome that is necessary to determine that the offspring will be male. Most of the rest of this tiny chromosome, the Y, harbors genes that are absolutely required for spermatogenesis in any species that has been shown to have a Y chromosome. Therefore, an individual with a defective Y chromosome, missing large chunks of its DNA, can still be a completely normal male, but cannot produce sperm. Uncovering how this Y chromosome and its corresponding X chromosome function has helped us understand the evolution of virtually all life on Earth. But the discussion of that incredible finding is complex and will require its own book. But I will give here a very simple summary.

During meiosis in the female ovary, when the woman's eggs are undergoing preparation for fertilization, all of her "like" (homologous) pairs of chromosomes, including her two X chromosomes, line up next to each other on the equatorial spindle plate in a uniform fashion. All of these like chromosomes, from 1 to 22, and also her two X chromosomes, adhere to each other and participate in this miraculous process called recombination. However, in the male testes, where sperm are being produced, the X and the Y chromosomes are so different that they cannot, and they do not, attach to each other or recombine along their entire length. Although homologous, they are really completely different chromosomes. The function of the SRY gene, located in the Y chromosome, is to determine, at six weeks of fetal development, that the indifferent gonad will turn into a testis (which begins the whole cascade of male rather than female development), and therefore it cannot get too intimate with the X.

But there is a tiny region at the tip of the Y chromosome that is identical to a similar region at the tip of the X chromosome, and at this tiny area near the tip, the X and the Y do line up, adhere to each other, and

recombine. (See fig. 12.17.) In this tiny little area, the X and the Y chromosomes function exactly as though they were autosomes. Without this residual pseudo-autosomal region at their tip, the X and the Y could never line up with each other and would never undergo proper meiosis. In other words, without this tiny pseudo-autosomal region, nondisjunction of either the X or the Y chromosome in the male testes would be routine. Without this tiny little area reserved for the X and the Y to be just like each other and to stick together in the process of meiosis during sperm production in the testes, most of the sperm so produced would have numerical errors in the number of sex chromosomes allotted to them. There would be sperm that had two X chromosomes instead of one, and there would be sperm that had two Y chromosomes instead of one. There would be sperm that had no X or no Y chromosomes. It is this fragile, tiny area of homology between the X and the Y, pulling them together to undergo proper meiosis, that prevents the majority of all our offspring from having what we call sex-chromosome aneuploidy.

Remember, we said that in 90 percent of cases, or more, autosomal trisomies (like trisomy 13, 18, or 21) come from female meiosis, from the mother's side, when her egg is being matured. Two copies of chromosome 21 are left in the egg after meiosis rather than the proper one copy of chromosome 21, owing to a faulty lineup of chromosome 21 on the spindle. This is caused by aging of the female's meiotic spindle.

Sex-chromosome aneuploidies, i.e., extra copies or missing copies of the X or Y chromosomes, are not associated with increasing age in either the male or the female, and the vast majority originate in the testes, from errors in meiosis during sperm production. The reason for such errors in sperm production resulting in sex-chromosome aneuploidy is the intrinsically fragile nature of that bond between X and Y chromosomes in that tiny little region called the pseudo-autosomal junction. That is why there is a higher incidence of sex-chromosome abnormalities in the offspring of men with extremely low sperm count.

Chromosomal Abnormalities in Infertile Men and Women, and in Their ICSI and IVF Children

Numerous studies of the chromosomes of infertile men with severely impaired sperm production and very low sperm counts have consistently demonstrated chromosomal errors in the blood samples of