

# The Y chromosome in the era of intracytoplasmic sperm injection: a personal review

Sherman J. Silber, M.D.

Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, Missouri; and Center for Reproductive Medicine, University of Amsterdam, Amsterdam, the Netherlands

The Y chromosome contains 60 multicopy genes composed of nine different gene families concentrated in regions of multiple repeat sequences called amplicons arranged in mirror images called palindromes. This pattern is susceptible to deletions caused by homologous recombination with itself, and can explain the presence of small numbers of sperm in otherwise azoospermic men. (*Fertil Steril*® 2011;95:2439–48. ©2011 by American Society for Reproductive Medicine.)

**Key Words:** Y chromosome, intracytoplasmic sperm injection, azoospermia, MESA, TESE

Almost concurrently with the development of intracytoplasmic sperm injection (ICSI) and testicular sperm extraction (TESE) for azoospermia in 1993, we began to study the genetic causes of male infertility via the first mapping of the Y chromosome in azoospermic males and in fertile control male populations, eventually leading to complete sequencing of the Y chromosome. This led to an understanding of why tiny amounts of sperm are often found in the testis of azoospermic men previously thought to be making no sperm (1–8). Subsequently, most of the research into the genetics of male infertility has been focused on aberrations of the Y chromosome. This specialized male chromosome, the Y chromosome, contains many genes that are involved in spermatogenesis, concentrated in a peculiar pattern of nucleotide repeats and mirror image inversions called amplicons and palindromes. Deletions involving these regions of the Y are found in 15% of severely infertile males and have been shown to be transmitted to male offspring via ICSI, presumably causing fertility problems in these children later in life (5, 9, 10).

The sequence of the Y also gives us a perspective about other male infertility genes that are widespread throughout the genome and which also could transmit infertility to future generations of ICSI offspring. More importantly perhaps, understanding the Y chromosome helps us to comprehend why men who are seemingly azoospermic often have some residual tiny amount of spermatogenesis that can be used for successful ICSI (11–13). Many genes on the Y chromosome required for spermatogenesis have multiple copies and even “backup” homologues elsewhere in the genome, such as *DAZL* and *CDYL* (14, 15). Another broader scientific benefit is that it has amplified our understanding of sex determination in general and has even helped to explain the origin of Turner syndrome (16). Indeed, studies of male infertility and the Y chromosome have gone as far as showing why XO female patients with Turner syndrome are generally caused not by the loss of an X chromosome in the female embryo, but rather by a loss of the Y chromosome in a male embryo.

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Reprint requests: Sherman J. Silber, M.D., Infertility Center of St. Louis, St. Luke's Hospital, 224 South Woods Mill Road, Suite 730, St. Louis, MO 63017 (E-mail: [silber@infertile.com](mailto:silber@infertile.com)).

## TREATMENT OF MALE INFERTILITY

Until 2 decades ago, there were no good treatment options for infertile couples when the male had severely impaired spermatogenesis. In fact, there are still no clinical therapies to correct deficient spermatogenesis (17–30). Since the introduction of TESE and ICSI by us and the Brussels Dutch-Speaking Free University in 1992, however, there has been a revolution in our thinking about male infertility (31, 32). Infertile couples with the most severe cases of male infertility, even with apparently 100% abnormal morphology and/or only rare spermatozoa in the ejaculate, can now have pregnancy and delivery rates not apparently different from conventional IVF with normal sperm (33–35).

In 1993, we first introduced microsurgical epididymal sperm aspiration (MESA) in conjunction with ICSI for the treatment of obstructive azoospermia (3, 12, 36–38). A few months later, TESE was also found to be effective for the majority of cases of nonobstructive azoospermia as well (3, 12, 13, 38, 39). The reason is that ~60% of azoospermic men with presumably no sperm production actually do have a minute amount of sperm production in the testis that is not quantitatively sufficient to spill over into the ejaculate, but which is adequate for ICSI (11, 13, 27, 39–46). Thus, even men with extremely low sperm counts, or with spermatogenesis so deficient in quantity that no sperm at all can reach the ejaculate, could now have children with the use of TESE-ICSI (3, 10, 47).

It is with these cases of nonobstructive azoospermia and severe oligozoospermia that the greatest concern has been registered for the fertility of future generations. If severe oligozoospermia or azoospermia is of genetic origin, then ICSI may create a potential problem of proliferation of male infertility in future generations (48). In fact, if one-half of all azoospermic men were to undergo ICSI, the incidence of male infertility would double within seven generations (Supplemental Fig. 1, available online at [www.fertstert.org](http://www.fertstert.org)) (49).

## GENETIC CAUSES OF MALE INFERTILITY: ROLE OF THE Y CHROMOSOME

Karyotyping reveals that structural chromosomal abnormalities, such as translocations and inversions, are found in about 1% of azoospermic men, but the most common karyotypic chromosomal

abnormalities in azoospermic men (2%) involve the sex chromosomes, such as in Klinefelter syndrome (47,XXY) (50–64). In the majority of these cases, surprisingly, we can usually find a few rare sperm adequate for fertilization using ICSI (44, 65–67). (See the [Supplemental Material](#) for more, available online at [www.fertstert.org](http://www.fertstert.org).)

Suspicion of involvement of the Y chromosome in male infertility actually arose originally from cytogenetic evidence reported more than 35 years ago (68). This karyotyping study showed grossly obvious terminal Y chromosome deletions in a very small percentage (0.5%) of azoospermic men who were otherwise phenotypically normal. It was then postulated that the Y chromosome contained a so-called *Azoospermia Factor* gene (*AZF*). During the middle 1990s, we showed that the long arm of the Y chromosome contains not one but many distinct deletion intervals and at least 60 genes belonging to nine gene families whose exclusive functions are in spermatogenesis (1–4, 6–8, 69). Interestingly, several of these multicopy spermatogenesis genes on the Y chromosome have “backup” homologues elsewhere in the genome, like *DAZL* on chromosome 3 and *CDYL* on chromosome 6.

In fact, the deletion frequency of one or more of these regions on the Y chromosome in men with azoospermia or severe oligozoospermia is ~15% (Fig. 1) (1, 3, 70). After our initial report, many laboratories throughout the world have reported on these submicroscopic deletions of the Y chromosome in azoospermic and severely oligozoospermic men (25, 69, 71–101). In fact, deletion screening of the Y chromosome is now considered standard practice for severely oligozoospermic and azoospermic patients undergoing assisted reproduction in most countries in the world.

### **AZFa**

The *AZFa* region differs from the *AZFb* and *AZFc* regions because of its nonrepetitive structure and its low deletion frequency (Fig. 2). *AZFa* deletions are very uncommon and only a few rare patients have been described (69, 79, 87, 102–104). However, studies of this region are highly useful in understanding the genetic basis of male infertility. The *AZFa* region spans ~800 kilobases (kb) and contains two functional single-copy genes: *USP9Y* and *DBY* (104, 105). This mutation represented the first and only case of a point mutation rather than a large microdeletion causing a single gene defect on the Y chromosome associated with spermatogenic failure (104). The lack of sequence repeats which characterize the rest of the Y chromosome made this particular region of the Y amenable to such a point mutation search. Because almost all other spermatogenesis genes on the Y chromosome, such as those in *AZFb* and *AZFc*, are multicopy, searching for point mutations in these genes is virtually impossible. It is also questionable whether point mutations in a single gene of a gene family will give rise to a severe infertility phenotype, because the remaining intact copies of the gene could potentially compensate for the loss of function of the mutated gene. This is an important lesson for why spermatogenic defects come in varying degrees of severity and why even in azoospermic men there are usually still a tiny number of healthy sperm “hiding” in the testes.

The *AZFa* region provides a good model for the interaction and overlapping functions of multiple genes and sheds light on the polygenic nature of the genetic control of spermatogenesis. When the entire *AZFa* region is deleted, taking out both *DBY* and *USP9Y*, the spermatogenic defect is severe and the patient is always azoospermic. In contrast, when only one gene is affected, such as loss of *USP9Y* function owing to a specific point mutation, this results in a less severe phenotype of maturation arrest with a few pachytene spermatocytes developing into a few mature sperm in some seminiferous

tubules. Thus, the loss of *DBY* (the only other gene in the *AZFa* region) likely exacerbates the spermatogenic consequences of the loss of *USP9Y*. (See the [Supplemental Material](#) for more).

### **AZFb**

Deletions of the *AZFb* region are slightly more common than deletions of the *AZFa* region but are still found in a very small percentage of azoospermic men (69, 95, 106–108). Interestingly, all men with deletions of *AZFb* described to date are azoospermic and show complete absence of spermatozoa in the testis (14, 69, 71, 81, 106, 107–111). Therefore, similarly to *AZFa* deletions, there are no reports on transmission of an *AZFb* deletion to ICSI offspring. Many functionally active genes are clustered within the *AZFb* region (81, 112, 113). Because of the presence of multiple sequence repeats within this region, in contrast to *AZFa*, efforts to define the *AZFb* region precisely had been hampered, but now the exact content and extent of the *AZFb* region has been elucidated and the entire Y chromosome has been fully sequenced (8, 106).

In fact, the region frequently referred to simplistically as *AZFb* overlaps *AZFc* and has a very large number of repeat copies of genes and pseudogenes (*RBMV*, *PRY*, *TTTTY*) arranged in a complex of palindromes (inverted repeat sequences). That is why we technically object to this commonly used terminology of “*AZFb*.” We prefer the more specific palindromic terminology. Deletions in this region of the Y chromosome are extensive and are much less common than in *AZFc*, and they are caused by weak “breakpoints” in the center of its inverted repeats. This is a less common deletion mechanism than that which plagues the *AZFc* region.

### **AZFc**

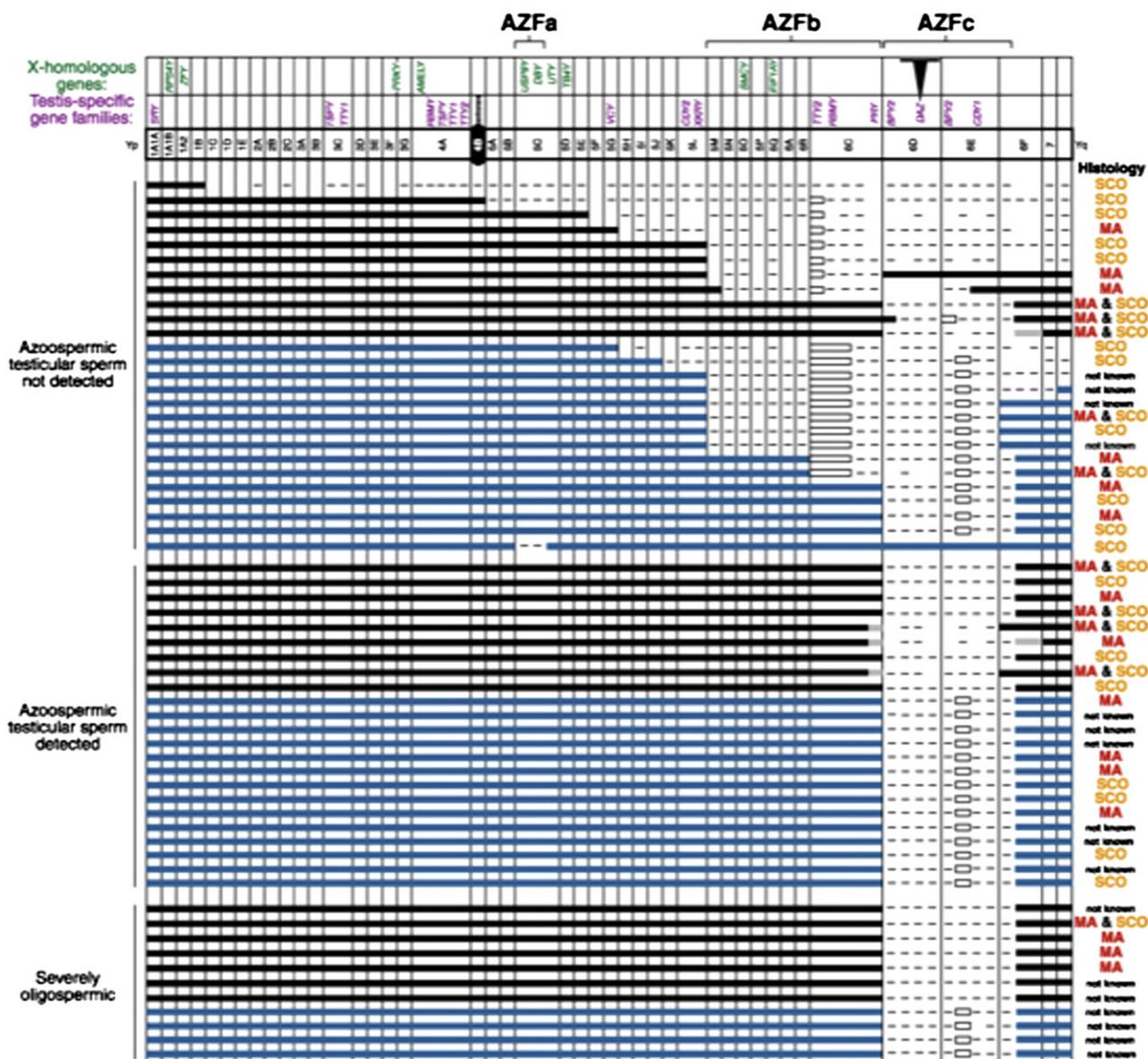
The most commonly deleted and best-studied region on the Y chromosome is the *AZFc* region. Deletion of the *AZFc* region is found in ~12% of azoospermic men and in 6% of severely oligozoospermic men (1–3, 70). The complete nucleotide sequence of the *AZFc* region reveals an extraordinary structure and genetic composition. The region is constructed from very large areas of absolute sequence identity called amplicons which are arranged in direct repeats, inverted repeats, or palindromes. The *AZFc* region spans 3.5 Mb (huge but not quite as huge as *AZFb* with its length of 8 Mb) and contains seven separate families of genes with a total of 19 genes that are all exclusively expressed in the testis (Fig. 3). This so-called “microdeletion,” *AZFc*, is actually a huge section of DNA, but not huge enough to show up with karyotyping. Interestingly, absence of this large 3.5-Mb *AZFc* chunk of the Y chromosome seems to have no other deleterious effect except upon spermatogenesis, exemplifying the remarkably specialized function of this region of the Y chromosome (2). These genes only affect spermatogenesis and nothing else.

The *DAZ* gene family, which is one of the seven gene families located within *AZFc*, was one of the first spermatogenesis genes identified on the human Y chromosome and comes in four almost identical copies (1, 6). The human *DAZ* genes were shown to be transcribed specifically in spermatogonia and in early primary spermatocytes (114). Interestingly, homologues of *DAZ* in other species were also shown to be involved in control of spermatogenesis, supporting an essential role of this gene in male fertility in humans as well as almost all other animals. Homologues of *DAZ* have been found in *Drosophila* (termed *boule*), in mice (termed *Dazl*), in frogs (termed *Xdazl*), and even in worms (termed *daz-1*) (115–120). Therefore, *DAZ* is the most ancient and well conserved spermatogenesis gene. In contrast to its presence in humans, the *DAZ* gene in these other species is single copy and located on an autosome rather than on the Y

**FIGURE 1**

Early map of major Y chromosome deletions in azoospermic men and the diversity of pathologic defects (3).

**Y Deletions and Diverse Histology in Azoospermic and Severely Oligospermic Men**



Silber. Spermatogenesis, Y chromosome, and ICSI. Fertil Steril 2011.

chromosome. In the human, *DAZ* is present on the Y chromosome in four near-identical repeat copies (99.9% homology) arranged in two clusters with two genes in each cluster (2, 7).

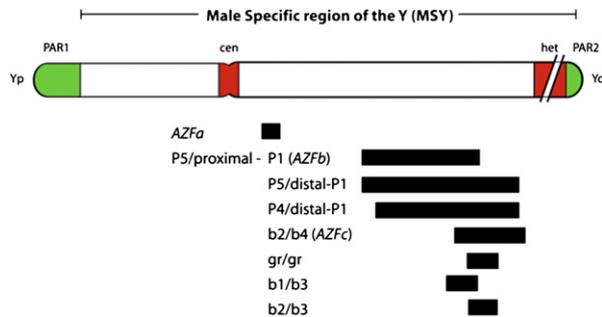
The human also retains an autosomal homologue of *DAZ* called *DAZL*, which is located on chromosome 3 (6). During evolution, some time after the split of Old and New World monkeys ~30 million years ago, the *DAZL* gene was transposed to the Y chromosome like a simple translocation event (Supplemental Fig. 2, available online at [www.fertstert.org](http://www.fertstert.org)). Once autosomal *DAZL* was transposed from chromosome 3 to Y, it was amplified and pruned until it became the modern-day *DAZ* gene family of four copies on the Y and still two autosomal copies on chromosome 3. In fact there is

yet another *DAZ* family homologue in humans, termed *BOULE*, that resembles the fly homologue *boule*, even more closely than *DAZ* or *DAZL* (121). The exact interaction and possible functional overlap between these three members of this interesting gene family holds the clue to the peculiar finding of a few surviving sperm in the testes of azoospermic men because of genetic “backup.” In fact, >80% of azoospermic men with an *AZFc* deletion have some sperm in the testis retrievable with TESE (4, 9, 10).

The *DAZL* gene on chromosome 3 was the original human *DAZ* gene, which jumped over to the Y chromosome 30 million years ago and resulted in our modern Y chromosome *DAZ* gene. The Y chromosome, with its lack of recombination during meiosis, represents

**FIGURE 2**

Simplified diagram of the main areas for Y chromosome microdeletions, including some that are less frequently studied, such as B<sub>2</sub>-B<sub>3</sub> (106).

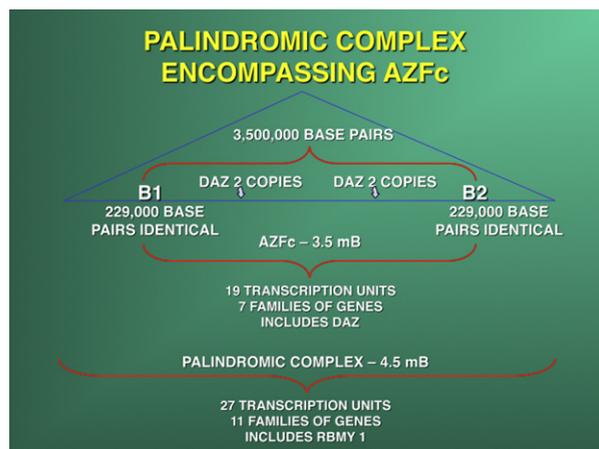


Silber. *Spermatogenesis, Y chromosome, and ICSI. Fertil Steril* 2011.

a safe harbor for the proliferation of genes that are beneficial to the male but detrimental to the female. This is how the Y chromosome, over evolutionary time, tends to accumulate genes from throughout the entire rest of the genome that control spermatogenesis. The Y chromosome in mammals, and other sex-determining chromosomes such as the W chromosome in birds, evolved differently over and over again in different genera, always with totally different genes and nucleotide sequences, but always with the same general theme: a degradation of its X homologues owing to failure of recombination (Supplemental Fig. 3, available online at [www.fertstert.org](http://www.fertstert.org)) and at the same time an accumulation and amplification of multiple copies of male-specific spermatogenesis genes from all over the genome. It is this multiplicity of gene copies all of which contribute quantitatively to total sperm production that explains the minute amount of sperm we can find at TESE in azoospermic men, because of “rescue” from the continued presence of at least one of these gene copies (such as *DAZL* on chromosome 3) even though most are missing or deleted.

**FIGURE 3**

Diagram of DNA sequence boundaries of *AZFc* deletion and the mechanism of *AZFc* deletion (2).



Silber. *Spermatogenesis, Y chromosome, and ICSI. Fertil Steril* 2011.

Deletions of the entire *AZFc* region result in loss of all four *DAZ* copies. Recent reports however, indicate that deletions involving only some of the *DAZ* genes are also found in infertile men, but these smaller deletions are found in men with only mild oligozoospermia, indicating a possible gene dosage effect, i.e., men with a deletion of only two *DAZ* genes are less affected than men with a deletion of all four copies (122–126). These are called GR-GR deletions, that is, half of *AZFc* is deleted. There are also less studied *AZFc* deletions, such as B<sub>1</sub>-B<sub>3</sub> and B<sub>2</sub>-B<sub>3</sub>. These data illustrate that infertility is a complex multigenic disorder and that disruption of different genes or disruption of some genes of a gene family can result in different degrees of spermatogenic failure.

## MECHANISM OF DE NOVO Y CHROMOSOME DELETIONS

Research into deletions involving *AZFa* and *AZFc* has provided interesting data on the mechanism of deletions, which sheds light on the inexorable decline in spermatogenesis in humans and in other species which have no “sperm competition.” Deletions on the Y chromosome are caused by “illegitimate” homologous recombination between highly similar or identical sequences which are found on the Y in great abundance. For example, homologous recombination between two identical sequence stretches results in dropout of the intervening *AZFa* region (105, 127, 128). Because these sequences of identical nucleotide repeats are shorter than those in *AZFc*, *AZFa* deletions are very rare.

However, for *AZFc* the substrates for homologous recombination are two repeats that are >99.9% identical and as long as 229 kb in length (2). The frequency with which these deletions occur seems to correspond to the length of the stretch of homology. Therefore, deletions of *AZFc*, caused by homologous recombination between 229-kb repeats, are far more common than deletions of *AZFa*, which are caused by repeats of only 10 kb in length. The very repetitive nature of the Y chromosome seems to be the cause of its instability over an evolutionary time frame, as well as in our current infertile male patient population, but it is also the method that the Y has adopted (albeit inefficient) for its survival, called “gene conversion” (129).

What is equally fascinating is how we survived at all as our X homologous genes on the Y chromosome deteriorated because of failure of meiosis with its nonrecombining mate (130, 131). In fact, this deterioration of X-homologous Y genes was the whole reason for the evolution of “X inactivation,” to put males and females on an equal footing despite different gene dosages.

## EVOLUTION AND GENETIC CONSTITUTION OF THE HUMAN Y CHROMOSOME

What makes the Y chromosome, with its confounding repeats, polymorphisms, and degenerating regions such an interesting object of study for male infertility? The answer lies in the evolutionary history of the X and Y chromosomes. Over the course of the past 240–320 million years of mammalian evolution, the X and Y chromosomes have evolved from what was originally a pair of ordinary autosomes (Supplemental Fig. 3) (14, 130–137). During that evolution, just as most of the ancestral X genes were decaying on the Y because of the lack of meiotic recombination, genes that control spermatogenesis arrived on the Y from autosomes. Once on the Y, these formerly autosomal genes amplified into multiple copies and achieved greater prominence through the process called “gene conversion” (6, 129, 138). Spermatogenesis genes that arrived on the Y, but came originally from autosomes, include the *DAZ* (from autosome 3) and *CDY* (from autosome 6) genes which are among the seven gene families located in *AZFc* (Supplemental Fig. 4,

available online at [www.fertstert.org](http://www.fertstert.org) (6, 15). Other spermatogenesis genes on the Y, such as *RBMY*, have persisted in their original position as on the X (139–142). The ancestral gene that remained on the X chromosome (*RBMX*) retained its widespread cellular functions, whereas *RBMY*, which persisted on the receding Y chromosome, evolved a male-specific function in spermatogenesis (141, 143–145). Male-benefit genes have thus arrived and accumulated on the evolving Y chromosome over many millions of years via three mechanisms: 1) persistence of genes on the ancestral X that evolved a male-specific function (*RBM* to *RBMY*); 2) retroposition from an autosome via reverse transcription (*CDL* to *CDY*); and 3) transposition from an autosome via translocation (*DAZL* to *DAZ*; Supplemental Fig. 2).

This evolution of the modern X and Y chromosomes was initiated by the emergence of a male sex-determining gene (now known as *SRY*) on what was originally an ordinary pair of autosomes (Supplemental Fig. 3) (15, 136, 137, 141). Genes associated with the nonrecombining *SRY* region that were specifically beneficial for male function or antagonistic to female function, flourished on the evolving Y chromosome despite the deterioration of more generalized genes that lacked the DNA repair benefits of meiosis (145–150).

The next question that the evolution of the X and Y chromosomes presents, which is crucial for understanding male infertility and ICSI, is how this degraded Y chromosome survives at all. The lack of recombination of the male-determining Y chromosome has led to complete deterioration and loss of most of its original 1,438 genes (the number of genes on its corresponding X) and an accumulation of only 60 genes (only nine gene families) which are male specific and are located in areas of sequence identity that promotes further deletions. So how does the Y survive at all, and why do we humans retain any spermatogenesis at all?

The answer is “gene conversion” (129). It answers the question of how these ampliconic repeats and palindromic inversions occur. When autosomes recombine during meiosis, DNA is exchanged in a way that the accumulated mutational errors of life get corrected in the germ cells via this DNA exchange. In a sense, the autosomes “have sex” with each other. This correctional meiosis cannot occur with the Y chromosome. Instead, the Y chromosome “has sex” with itself. That is, the like-sequence repeats of the Y chromosome “recombine,” in a sense, with each other, an “illegitimate” homologous recombination. This “gene conversion” creates and repairs the multiple copies and inverted DNA sequence repeats that characterize the Y and indeed all sex-determining chromosomes. So if there is a missing or deleted copy of a spermatogenesis gene, there are other backup copies that can still rescue spermatogenesis to some degree.

Throughout our whole study of Y deletions, the most confusing was the so-called “terminal deletion,” shown in the top six cases of Figure 1. With molecular analysis, they appeared with initial mapping not to be interstitial deletions, but rather “isodicentric,” meaning that the Y chromosome did not just come to an end point proximally, but rather was doubled over on itself with like copies annealed to each other. This is caused by a kind of “gene conversion” that went awry. (See the Supplemental Text for more.) Thus, “gene conversion” as a mechanism for maintenance and repair of the Y chromosome has more limited value than the standard meiosis that the X chromosome in the ovary and the autosomes enjoy.

## THE Y CHROMOSOME AND SPERMATOGENESIS IN HUMANS AND IN APES

Comparing spermatogenesis in humans, chimpanzees, and gorillas has always been fascinating (52, 149). Chimpanzees, which weigh

only about 100 pounds, have enormous 8-cm-diameter round (not oval) testes with sperm counts of >1 billion/mL. Yet gorillas, which weigh as much as 600 pounds or more, have tiny testes, very poor spermatogenesis, and, in the sparse literature on gorilla testicular histology, in the majority of cases have what appears to be Sertoli cells only (151). Humans, the closest living relatives to chimpanzees and gorillas, fall somewhere in between.

Most intriguing is to compare the human Y chromosome to the chimpanzee Y chromosome, both of which have been fully and accurately sequenced (8, 152). Unfortunately, the gorilla Y has not yet been sequenced. Some interesting differences are noted between the human and chimpanzee Y chromosomes (152). The chimpanzee Y chromosome has many more amplicons and palindromes than the human, but it nonetheless has much fewer ampliconic genes (25 compared with 60). So the increased sperm production of chimpanzees cannot be explained by the presence of more testis-specific genes on their Y chromosome. But interestingly, the chimpanzee Y is missing a gene (*PRY*) that is present on *AZFc* in two copies in the human. You cannot attribute the vast superiority of chimpanzee spermatogenesis to having more copies of spermatogenesis genes. But the *PRY* gene, which humans have and chimpanzees do not have, could be a suppressor of spermatogenesis. Thus, a deletion of *PRY* in the chimpanzee could conceivably be a reason for its huge amount of spermatogenesis. Furthermore, it is fascinating that this gene has also been found to be present in only one copy in rare humans who have incredibly high sperm counts, approaching half a billion (153). Thus, comparing the superfertile chimpanzee Y chromosome to its less fertile human cousin, can help us to better understand the genetic control of spermatogenesis in our infertile male patients.

## OTHER MALE INFERTILITY GENES NOT ON THE Y CHROMOSOME

Although most research into the genetics of male infertility has focused on the Y chromosome, many other spermatogenesis genes are present throughout the human genome. Recent studies and earlier speculations indicate that the Y chromosome is not the only chromosome that accumulates genes that benefit spermatogenesis over an evolutionary time span (133, 148, 154, 155). Its counterpart, the X chromosome, also seems to be an ideal locus for spermatogenesis genes. Like the Y chromosome, the X chromosome is present as a single copy in the heterogametic XY male. Of course, in the egg, i.e., in the female, the X chromosome undergoes normal meiosis and repair with its paired X mate. However, the X chromosome, like the Y chromosome, does not get to undergo normal meiotic repair in the testis. So it cannot escape completely some of the problems that the Y chromosome faces. Therefore, the X chromosome also has large ampliconic regions. Furthermore, any genetic alteration on the X would have an immediate impact in the male, because there is no other X chromosome to compensate or counteract the effect. Therefore, a recessive gene mutation on the X that is beneficial to the male would preferentially be allocated to the X chromosome as opposed to an autosome. The X chromosome (which is only repaired at meiosis in the egg, not in the testis) has huge ampliconic regions, similarly to the Y chromosome, which have not yet been well characterized and should have a rich complement of spermatogenesis genes.

In fact, reverse-transcription PCR subtraction studies of spermatogonia in mice have demonstrated that a large fraction of genes that are expressed exclusively in premeiotic male germ cells are indeed X chromosomal in origin (155). Eleven of the 36 genes expressed specifically in mouse spermatogonia were found exclusively on the

X chromosome. Because the X chromosome is well conserved in all mammals, it seems very likely that evolution has also conferred on the human X chromosome a large portion of the burden for spermatogenesis. (See the [Supplemental Text](#) for more).

### TRANSMISSION OF Y DELETIONS TO ICSI OFFSPRING

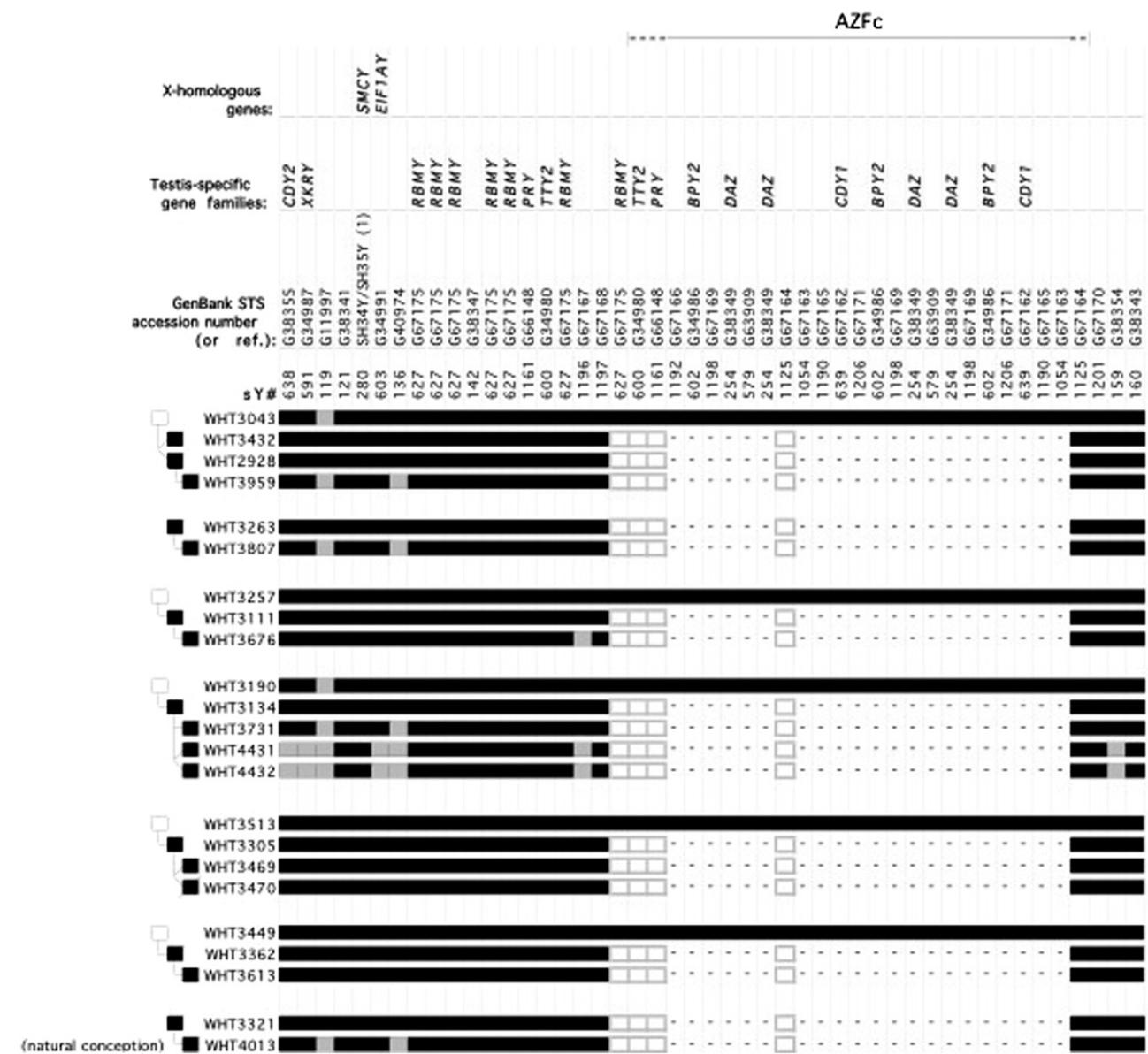
Once it had been shown that many cases of male infertility were caused by deletions on the Y chromosome, concern was registered immediately about the possibility of transmitting these deletions (or other genetic causes of male infertility) to offspring via what was the relatively new technique of ICSI, first introduced in 1992. Although the first boys born from ICSI procedures are not yet 20 years old, it seems likely that if they carry the same genetic defect

as their father on their Y chromosome, they will be found to be infertile just as their fathers were.

Microdeletions on the long arm of the Y chromosome do not appear to adversely affect the fertilization or pregnancy results in either severely oligozoospermic or azoospermic men, from whom sperm was successfully retrieved by TESE (3, 9, 10). Concern had been registered that ICSI results might be poorer in Y-deleted men, but this has not been the case (10, 101). Thus, men with Y deletions have the same chance of obtaining offspring via ICSI as non-Y-deleted males undergoing ICSI. Most genes involved in these Y deletions are expressed specifically in the testis during spermatogenesis but do not seem to be essential for fertilization or embryogenesis. The only problem for obtaining a pregnancy is the

**FIGURE 4**

Y deletions are transmitted to all male intracytoplasmic sperm injection (ICSI) offspring. The top lines show the normal Y chromosome of the infertile male's father. The next lines show the AZFc deletions of the infertile male and the identical AZFc deletions of his male ICSI offspring (9).



Silber. Spermatogenesis, Y chromosome, and ICSI. *Fertil Steril* 2011.

low number of sperm available in these patients, and this is circumvented by the ICSI procedure.

There has been some concern expressed about a possible widening of the *AZFc* deletion when it is transmitted to the next generation, but in our centers all of the male offspring from Y-deleted men have had the same Y deletion as their infertile father without any expansion (5, 9, 125, 156). In fact, there was no cogent reason to expect there would be any expansion, considering the mechanism of these Y deletions. So we also examined the Y chromosome of fathers, brothers, and paternal uncles of the infertile men for Y deletions and fertility. In all of the infertile Y-deleted men, the deletions were shown to be completely new, i.e., the fertile fathers of the infertile Y-deleted patients had no Y deletion, the deletion first appearing in the infertile sons. However, all male offspring of these infertile Y-deleted men derived from ICSI procedures had the same Y deletion of their father transmitted to them without any change (Fig. 4) (9). So there is no evidence that the male offspring will be any worse off than his father. Therefore, most couples with male infertility who have a baby are not very troubled by the possibility that their son might have a similar problem (157, 158). (See the Supplemental Text for more).

It can therefore be assumed that most Y chromosome deletions arise during spermatogenesis in the fertile testis of the infertile man's fertile father, and not during embryogenesis. The deletions that arise in the testis of the fertile father are caused by an accidental homologous recombination between large sites of sequence identity causing loss of the intervening sequence. The exact frequency of the occurrence of these deletions in the testis is unknown, although it is estimated that ~1 in 1,000 newborn boys are Y-deleted (2). The mechanism for the occurrence of Y deletion in a boy is that 1 out of every 1,000 or so sperm produced in every normal male's testis

has a Y deletion caused by illegitimate homologous recombination. (See the Supplemental Text for more).

## CONCLUSIONS ABOUT Y DELETIONS AND ICSI

The use of ICSI has increased tremendously over the past 2 decades and currently allows even azoospermic men with only minute amounts of spermatozoa in their testis to father children. In the majority of cases, there is no obvious explanation for the deficient sperm production in the male other than genetic, and it is possible that male ICSI offspring would inherit the aberration and therefore also be infertile. However, this possibility has rarely deterred the infertile couple from undergoing ICSI. Studies on the role of Y deletions in male infertility have provided new insights into this transmission of male infertility via ICSI and greater understanding of why we can find some few sperm in the testes of azoospermic men. Through evolution, the Y chromosome acquired a specialized role in spermatogenesis, and this has made it highly useful in studying the genetic causes of male infertility. Aberrations on the Y chromosome are currently found in ~15% of severely infertile men. Most, though not all, of these men still possess some degree of spermatogenesis that results in sufficient spermatozoa to perform ICSI and have children. The presence of Y deletions does not decrease the fertilization or pregnancy rate with ICSI, thereby enabling these men to father children with the same efficiency as non-Y-deleted men undergoing ICSI.

It is apparent that there is likely to be frequent transmission of male infertility from the ICSI father to his male offspring, regardless of current Y chromosome testing. Every couple must decide for themselves whether they wish to consider this risk. In our experience, almost all such couples, when well informed, still choose to have ICSI despite this risk (159). Parents usually conclude that if they can have a baby via ICSI, then their male offspring also can.

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## SUPPLEMENTAL MATERIAL

### Genetic Causes of Male Infertility: Role of the Y Chromosome

However, the percentage of male infertility that can be explained by karyotyping alone is low, because of the low resolution of cytogenetic studies. It was not until the development of modern molecular techniques such as polymerase chain reaction (PCR) that the genetic causes of male infertility could be studied with much greater detail. Since then, many more genetic abnormalities, such as microdeletions and point mutations, have been described in infertile males, with most research focusing on the role of genes on the human Y chromosome. Many cases of male infertility are still diagnosed as idiopathic, but they are likely to prove to be genetic in origin also.

#### *AZFa*

This finding in the *AZFa* region runs parallel to our previous observations of spermatogenesis in that larger Y deletions (which take out more genes) are associated with a lesser likelihood of finding sufficient sperm for ICSI than smaller deletions (3, 106) (references are as provided in the original print article).

### Evolution and Genetic Constitution of the Human Y Chromosome

As we have explained, massive palindromes, or mirror-image inverted repeats of DNA sequence, characterize the “ampliconic” portion of the Y chromosome, which contains all the testis-specific genes. In the absence of meiotic pairing, these gene pairs are maintained by intrapalindrome arm-to-arm recombination. This is like the Y chromosome having to pair with itself, because it cannot pair as in normal meiosis to any homologous (or like) chromosome.

But this gene conversion mechanism can result in crossing over between sister chromatids to form instead these isodicentric double strands which just appear to be terminal deletions. This certainly results in spermatogenic failure because of missing distal genes, but there is a double copy of the proximal part of the chromosome. The bigger problem is that the Y chromosome now contains two centromeres, which results in meiotic instability. The farther away the centromeres are from each other, i.e., the longer the isodicentric Y chromosome, the greater will be the chance that the entire chromosome will be deleted, resulting in an XO female Turner embryo, rather than what should have been a sterile male.

### The Y Chromosome and Spermatogenesis in Humans and in Apes

The generally accepted reason for this massive discrepancy in spermatogenesis between these three closely related species lies in their differing mating patterns. Chimpanzees congregate in troupes of 30 to 40 in an extended family wherein any female who goes into heat is instantly mounted by every single male in the troupe. Therefore, there is an intense “competition” among the sperm of the different males for which one will fertilize the eggs of the females. It is far more likely that the male with the highest sperm count, and the biggest testicles, will become the father of the male offspring, because of this high degree of “sperm competition” in chimpanzees.

In gorillas, it is the opposite. Any female is permanently attached to just one single silverback alpha male, and if she ever gets pregnant, it will have to be with his sperm only. So, in gorillas, there is no sperm competition. That results in small testes with very low

sperm counts in these otherwise huge, very macho animals. But why is that? The answer lies in the peculiar instability of the nonrecombining Y chromosome (149).

The multiple nucleotide direct and inverted sequence repeats (amplicons and palindromes) are where all the testis-specific spermatogenesis genes on the Y are located. These areas are prone to frequent deletions caused by nonhomologous, or “illegitimate” homologous recombination with itself, as already explained, resulting in dropouts of often huge chunks of DNA, causing the concentration of spermatogenesis genes on the Y chromosome to have a very fragile existence. So without sperm competition, sperm counts over eons of time are likely to go down.

### Other Male Infertility Genes Not on the Y Chromosome

Future research into genes on the human X chromosome (not just the Y) and their role in the male will provide even more insights into the genetic basis of male infertility. Beyond the X and Y chromosomes, whole-genome sequencing on all of our original study participants may eventually reveal many other testis-specific genes scattered throughout the genome.

### Transmission of Y Deletions to ICSI Offspring

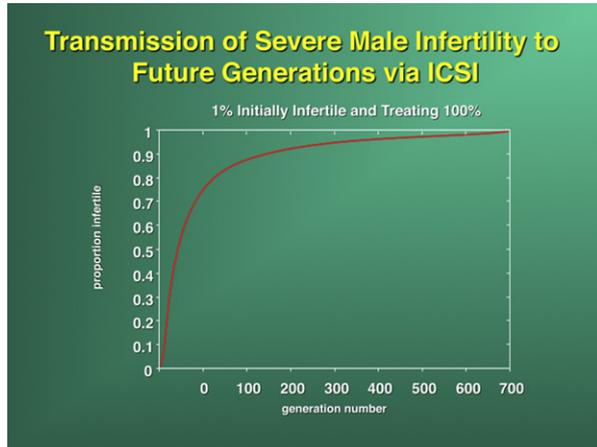
This is not the case for the testes of sons derived from fathers with XXY Klinefelter syndrome. These offspring all have a normal XY karyotype and are likely to be fertile, whereas spermatozoa from *AZFc*-deleted men carry the same deletion that was originally detected in their somatic cells (156). Thus, it seems that if a patient carries a deletion on the Y chromosome, as determined by analysis of his blood, all of his spermatozoa will have the same deletion, as will all of his sons when these spermatozoa are used for ICSI.

The result of deletions on the Y chromosome is impaired spermatogenesis and not necessarily a failure to father offspring naturally. The degree of impairment, i.e., mild oligozoospermia, severe oligozoospermia, or azoospermia, differs between different deletions and between different patients with the same deletion. Whether these men are able to induce pregnancies depends on the fertility of their sexual partners. The fact that even men with *AZFc* deletions can, in some instances, father children without the use of assisted reproduction technologies, illustrates the ability of some wives of men with minute amounts of spermatozoa to conceive naturally (10, 94, 158). Because some deletions, such as partial deletions of the *DAZ* clusters, potentially have a less dramatic impact on spermatogenesis, these could very well be transmitted naturally more often (126). However, the use of ICSI could contribute considerably to the spreading of these deletions and, as a consequence, reduce the overall fertility within the human population (Fig. 1) (49).

If a genetic aberration that leads to male infertility is located not on the Y, but on an autosome or on the X chromosome, the possible transmission of this aberration is different from transmission of aberrations on the Y chromosome. In contrast to aberrations on the Y chromosome, aberrations on the X chromosome are never transmitted to male offspring, because all boys inherit an X chromosome from their mother only. All girls, however, born from these men with X chromosome aberrations are carriers. Because these women are not affected by the aberration (because it affects only testis-specific genes) and are therefore fertile, they can naturally transmit the aberration to their offspring, giving rise to infertility in the second generation in 50% of boys and causing 50% of girls to be carriers.

## SUPPLEMENTAL FIGURE 1

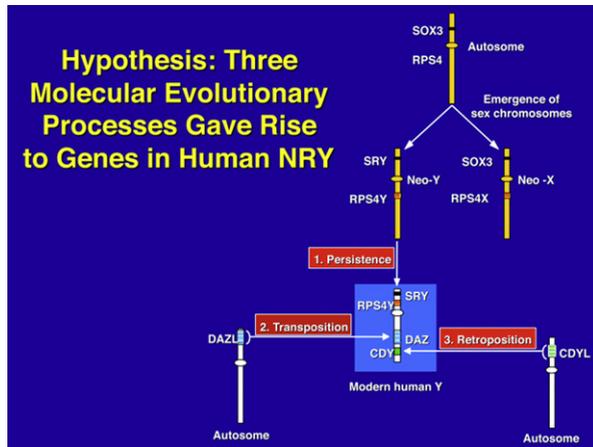
A theoretical model showing that eventually the sperm count will continue to go down and reach zero in 10,000 years because of intracytoplasmic sperm injection (ICSI) (49) (references are as provided in the original print article).



Silber. Spermatogenesis, Y chromosome, and ICSI. *Fertil Steril* 2011.

## SUPPLEMENTAL FIGURE 2

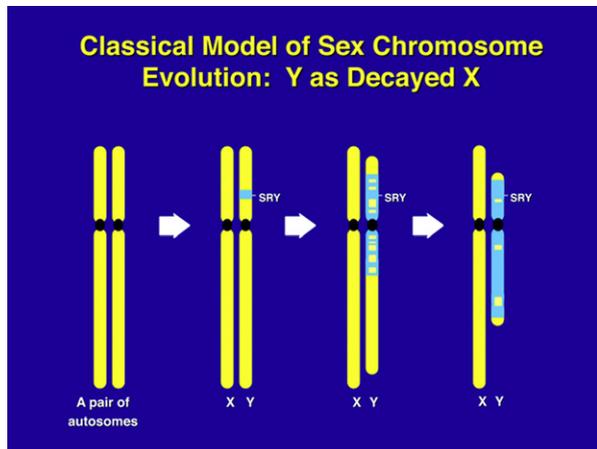
Diagram showing the evolutionary accumulation of male-specific genes to the Y chromosome by transposition, retroposition, and persistence (15, 138).



Silber. *Spermatogenesis, Y chromosome, and ICSI. Fertil Steril* 2011.

## SUPPLEMENTAL FIGURE 3

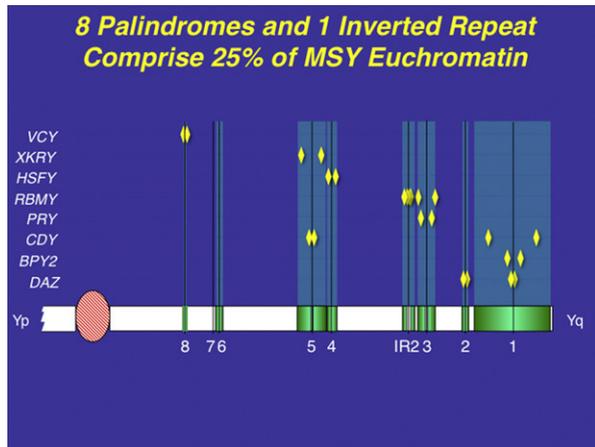
Evolution of Y chromosome from what was originally a pair of ordinary autosomes 300,000,000 years ago.



*Silber. Spermatogenesis, Y chromosome, and ICSI. Fertil Steril 2011.*

## SUPPLEMENTAL FIGURE 4

All “male-benefit” spermatogenic genes on the Y chromosome have accumulated in ampliconic regions of repeat DNA sequences and inverted repeat DNA sequences (8) (references are as provided in the original print article).



Silber. *Spermatogenesis, Y chromosome, and ICSI. Fertil Steril* 2011.