In: Essential IVF 2003
(eds. van Blerkom, J. and Gregory, L.) Kluwer Academic Publishers, Boston, MA

CHAPTER FIVE

GENETICS OF MALE INFERTILITY: EVOLUTION OF THE X AND Y CHROMOSOME AND TRANSMISSION OF MALE INFERTILITY TO FUTURE GENERATIONS

Sherman J. Silber

Infertility Center of St. Louis
St. Luke's Hospital
224 South Woods Mill Road, Suite 730
St. Louis, MO.

THE USE OF ICSI IN AZOOSPERMIC AND OLIGOSPERMIC MEN: INTRODUCTION TO THE PROBLEM

Since the introduction in 1992 of intracytoplasmic sperm injection, there has been a revolution in our thinking about male infertility (Palermo et al., 1992; Van Steirteghem et al, 1993). The most severe cases of male infertility, even with apparently 100% abnormal morphology, and even just rare sperm in the ejaculate, could now have pregnancy and delivery rates apparently no different from conventional IVF with normal sperm (Nagy et al, 1995; Liu et al., 1994; Liu et al., 1995). In 1993, testicular sperm extraction (TESE) and microsurgical epididymal sperm aspiration (MESA) in conjunction with ICSI was introduced for the treatment of obstructive azoospermia (Schoysman et al., 1993; Devroey et all, 1994; Silber et al., 1994, 1995a; Tournaye et al., 1994; Devroey et al., 1995a). Eventually this technique was also used for "non-obstructive" azoospermia (Devroey et al., 1995b; Silber et al., 1995b, 1996, 1998a). Many azoospermic men have a minute amount of sperm production in the testis that is not quantitatively sufficient to "spill over" into the ejaculate, but is adequate for ICSI (Silber et al., 1995b, 1995c, 1997a, 1997b; Silber and Rodriguez-Rigau, 1981; Steinberger and Tjioe, 1968; Zukerman et al., 1978). It is with these cases of non-obstructive azoospermia and severe oligospermia that the greatest concern has been registered for the well-being of offspring generated by ICSI. Thus, if severe oligospermia or azoospermia is of genetic origin, in many cases, ICSI creates a potential problem of proliferation of male infertility (Silber 1998b; Faddy et al., 2001).

The purpose of this chapter is firstly to explain the accumulating molecular data on Y chromosomal spermatogenesis genes, and their transmission to

ICSI offspring. The second purpose is to outline the reasons for concentrating on the evolution of the Y chromosome, and the light it sheds on the existence of *many more spermatogenesis genes* that are widespread throughout the genome, and that may also be responsible for transmitting male infertility to future generations. A third, and simpler, goal is to review the more routinely appreciated cytogenetic aspects of male infertility, and its impact on ICSI offspring.

EARLY GENETIC STUDIES OF AZOOSPERMIC AND SEVERELY OLIGOSPERMIC MEN

For several decades, it had been speculated that there was a genetic etiology to many cases of male infertility (Silber et al., 1995b; Silber 1989). This suspicion originally arose from cytogenetic evidence reported over 25 years ago in a very small percentage (0.2%) of azoospermic men who were otherwise phenotypically normal, but who had grossly obvious terminal Y chromosome deletions (Fig. 5.1A,B) (Tiepolo and Zuffardi, 1976).

Simple karyotyping of infertile men also raised the possibility of infertility being associated with autosomal translocations (Van Assche et al., 1996; Bonduelle et al., 1995, 1996, 1998a, 1998b, 1999; Egozcue et al., 2000). A massive summary of karyotyping results in newborn populations, reviewed by Van Assche, revealed an incidence of balanced autosomal translocations in a normal newborn population of 0.25% but an incidence of 1.3%, in infertile men (Table 5.1) (Van Assche et al., 1996). In fact, karyotyping of oligospermic males (i.e. less than 20 million per cc) reveal a 3% incidence of some type of autosomal chromosome anomaly, either balanced Robertsonian translocations, balanced reciprocal translocations, balanced inversions, or extra markers. These translocations could conceivably be transmitted to offspring if ICSI allowed them to conceive. However, because of the limitations of the resolution of cytogenetics, and the very small percentage of these readily discernable karyotypic abnormalities found in infertile men, until recently it had been a convoluted struggle to study the genetic causes of male infertility, and the possible transmission of these genetic errors to the offspring of couples with male infertility (Egozcue et al., 2000).

The possibility that many more cases of male infertility might be genetic was bolstered by the failure of most clinical therapies to correct deficient spermatogenesis (Devroey et al., 1998; Baker et al., 1981; Baker et al., 1984, 1985; Baker and Kovacs, 1985; Baker 1986; Nieschlag et al., 1995, 1998; Nilsson et al., 1979; Rodriguez-Rigau et al., 1978; Schoysman 1983; Silber et al., 1995b; Silber 1989). The heritability of sperm count demonstrated in the wild (O'Brien et al., 1986, 1987; Short 1995), classic studies of naturally occurring pure sterile Y deletions in Drosophila, and very early molecular investigations of the Y chromosome in humans led to what has now become an intense search for genes which control spermatogenesis and which may be

defective in many or most infertile males (Johnson et al., 1989; Ma et al., 1992, 1993; Eberhart et al., 1996; Hockstein et al., 1995). However, only recently has the frequent genetic etiology of male infertility related to defects in spermatogenesis (not to mention obstruction) become widely acknowledged via molecular methodology (Kent-First et al., 1996; Kremer et al., 1997, 1998; Krausz and McElreavey, 2001; Silber et al., 1995b; Vogt 1996, 1997; Reijo et al., 1995; Chillon et al., 1995; Shin et al., 1997; Anguiano et al., 1992). If male infertility is of genetic origin, its possible transmission to offspring of successfully treated infertile men is a serious social concern (Page et al., 1999; Mulhall et al., 1998; Silber 1998b; Faddy et al., 2001).

TABLE 5.1 PERCENTAGE OF CHROMOSOME ABNORMALITIES OBSERVED IN SEVEN SERIES OF INFERTILE MEN (AZOOSPERMIC AND OLIGOSPERMIC) COMPARED TO NORMAL NEWBORN POPULATION

All References	Number	Sex Chromosomes	Autosomes	Total
Total	7,876	295 (3.8)	104 (1.3)	399(5.1)
Newborn Infants	94,465	131 (0.14)	232 (0.25)	366 (0.38)

Van Assche et al., 1996

Y CHROMOSOME MAPPING OF INFERTILE MEN AND ICSI

With simple karyotyping, it has been known that a very small number of azoospermic men (0.2%) have large defects visible in the long arm of the Y chromosome that are not present in their fertile fathers. This implied the existence of an azoospermic factor somewhere on Yq. (Tiepoloand Zuffardi, 1976). However, smaller defects (i.e. "microdeletions") could not be discerned with those limited early cytogenetic methods (Fig. 6.1,B). Therefore, these defects in Yq were considered to be rare even in azoospermic men.

In 1992, comprehensive Y chromosomal maps were constructed using yeast artificial chromosomes (YACS) and sequenced tagged sites (STS), and this created the possibility for more detailed study of the Y chromosome in infertile men (Foote et al., 1992; Vollrath et al., 1992). Using polymerase chain reaction (PCR), a more refined search for Y chromosome deletions could be pursued by testing for as many as 52 DNA landmarks (STSs, or sequence tagged sites) across the entirety of the Y chromosome. All Y-DNA markers employed were placed on a physical map of the chromosome, the

markers representing all gene families that were then known in the non-recombining region of the Y chromosome (Vogt et al., 1997; Foote et al., 1992; Vollrath et al., 1992; Lahn and Page, 1997). Using these molecular mapping techniques, which have much greater resolution than cytogenetics, a large series of severely infertile men with clearly identified phenotypes revealed deletions in 13% of azoospermic males (Reijo et al., 1995) (Fig. 5.2).

As many as 7% of severely oligospermic men also had these same "microdeletions" (Silber et al., 1998; Reijo et al., 1996). The most commonly deleted region was located in the distal portion of interval 6, subsequently referred to as AZFc (Fig. 5.3A-C) (Silber et al., 1998; Vogt et al., 1996; Reijo et al., 1995). The higher resolution of Y mapping over karyotyping thus showed that more than just 0.2% of azoospermic men had defects of the Y chromosome, and more than just a few percent of severely infertile men had a genetic cause for their condition. However, because of the highly polymorphic nature of the non- recombining region of the Y (NRY), there are many Y deletions that are of no consequence. Only if these deletions in the

FIGURE 5.1. A AND B. Karyotype of the azoospermic male with cytogenetically visible Yq deletion compared to karyotype of an azoospermic male with a normal Y chromosome.

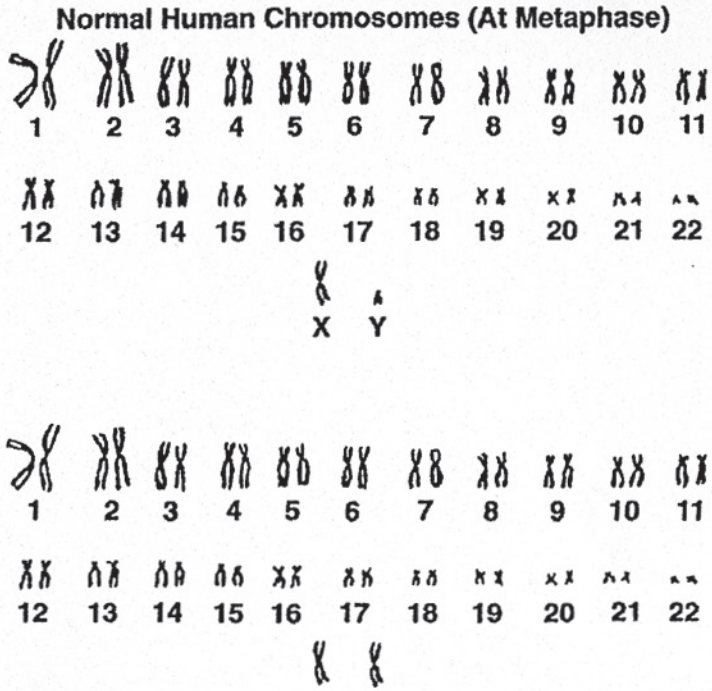


FIGURE 5.1C. Normal male karyotype compared to normal female karyotype.

infertile male are not present in his fertile male relatives, nor in hundreds of normal controls, could they be implicated as a cause of the infertility. The fertile fathers of the Y-deleted, infertile men were shown to have intact Y chromosomes, demonstrating that the deletions had arisen de novo and providing strong evidence that these de novo deletions were indeed the cause of the spermatogenic failure observed in these men. Many laboratories throughout the world have reported on these sub-microscopic deletions of the Y chromosome in azoospermic and severely oligospermic men (Vogt et al., 1996, 1997; Pryor et al., 1997; Ma et al., 1993; Girardi et al., 1997; Mulhall et al., 1997; Kremer et al., 1997; Vereb et al., 1997; van der Ven et all, 1997; Foresta et al., 1997; Chai et al., 1998; Elliot et al., 1997; Nakahori et al., 1996; Qureshi et al., 1996; Najmabadi et al., 1996; Simoni et al., 1997; Bhasin et al., 1994; Kent-First et al., 1996, 1999; Morris and Gleicher, 1996; Krausy and McElreavey, 2001; Chang et al., 1999; Cram et al., 2000; Grimaldi et al., 1998; Kim et al., 1999; Krausyet al., 1999; Liow et al., 1998; Oliva et al., 1998; Seifer et al., 1999; Stuppia et al., 1998; Van Golde et al., 2001;

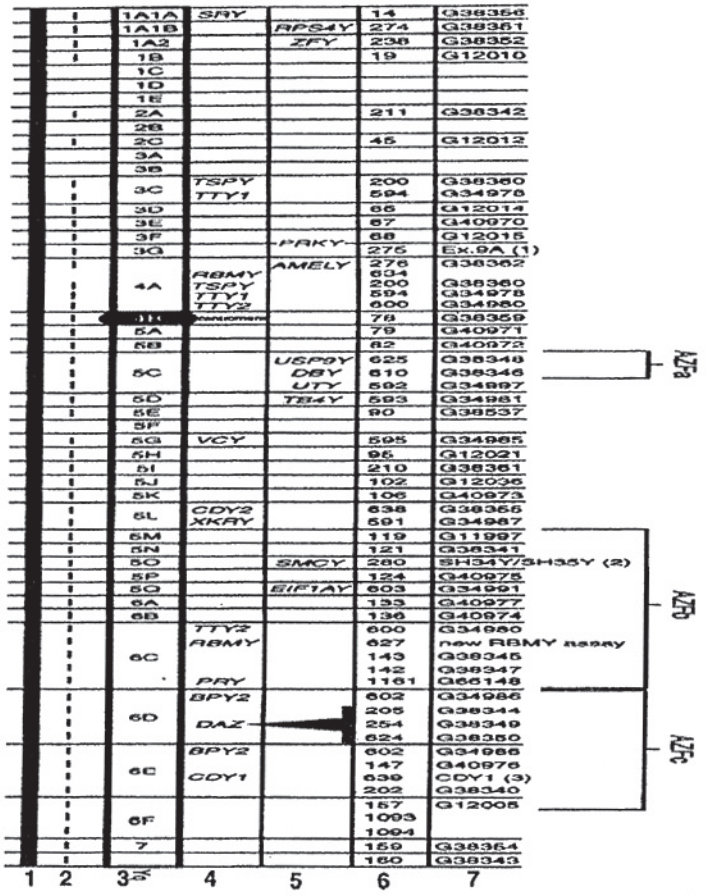


FIGURE 5.2. Y chromosome map based on STS interval markers and their corresponding X-homologous and testis-specific gene. 1. Fertile male control; 2. Fertile female control,; 3. Yp; 4. Testis-specific gene families; 5. X-homologoous genes; 6. SY#; 1. GenBank STS accession number (or ref.).

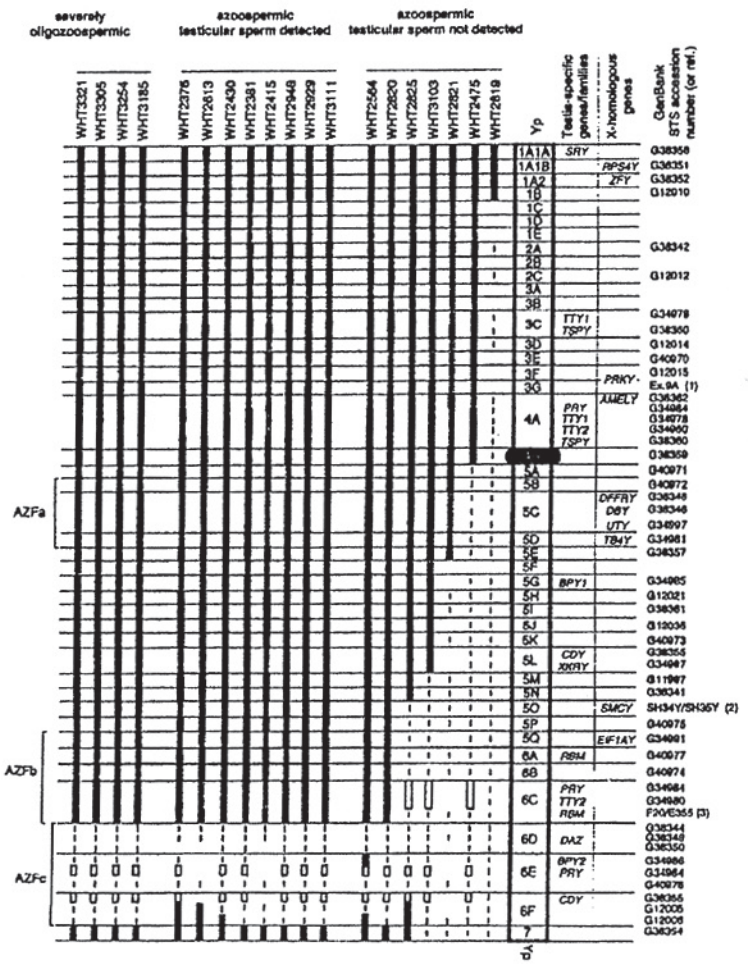


FIGURE 5.3A. Typical early deletion map of azoospermic and severely oligospermic men with chromosomal microdeletion.

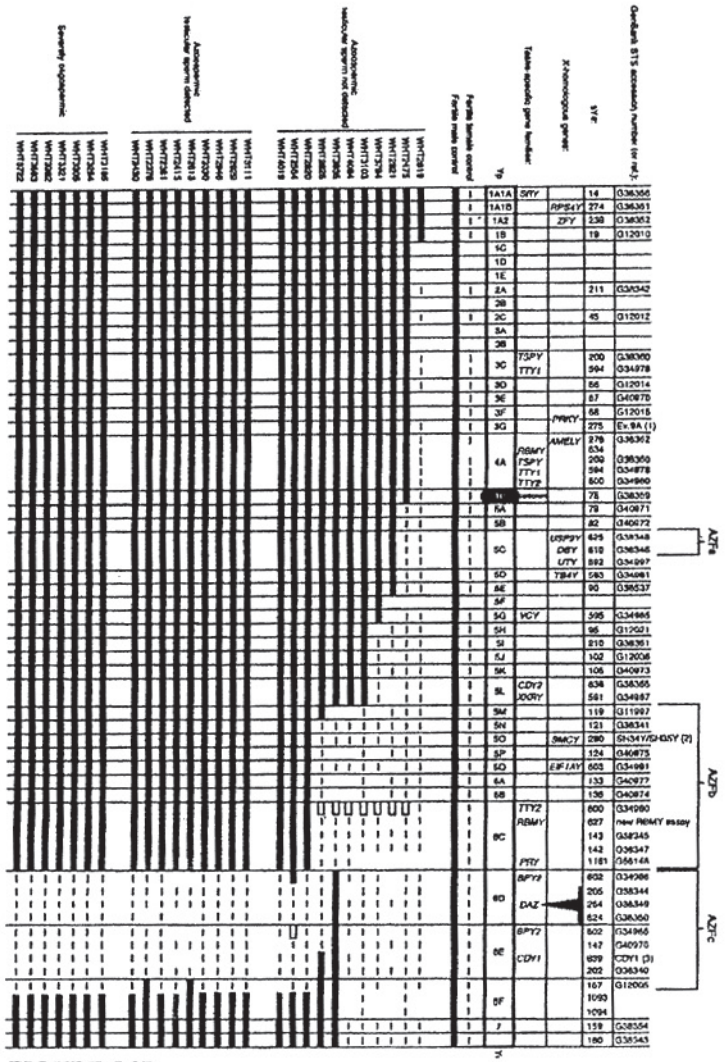


FIGURE 5.3B.

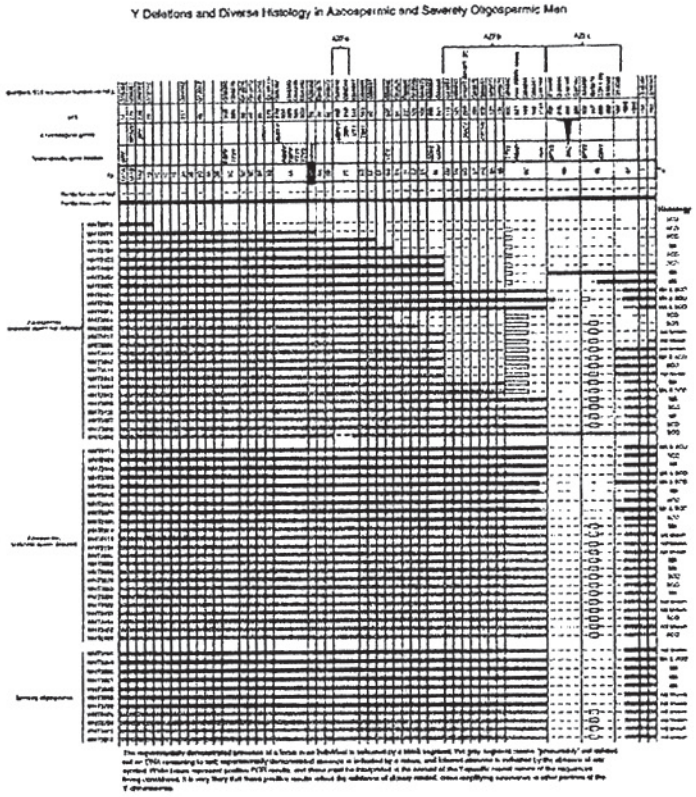


FIGURE 5.3B AND 3C. More refined, later deletion maps of azoospermic and severely oligospermic men.

Studying AZFa also provided a good model for the interaction and overlapping functions of multiple genes which 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, there is a more severe spermatogenic defect and the patient is azoospermic. However, when there is only a specific point mutation of the USP9Y gene, we observed maturation arrest with a few pachytene spermatocytes developing into mature sperm in a few seminiferous tubules. Thus, the loss of DBY (the only other gene in the AZFa region) exacerbates the spermatogenic consequences of the loss of

USP9Y. This finding in the AZFa region runs parallel to previous observations that larger Y deletions (which take out more genes) are associated with a lesser likelihood of finding sufficient sperm for ICSI (Silber et al., 1998).

TABLE 5.2 GENES OF THE DAZ FAMILY IN VERTEBRATES AND DROSOPHILA

	Y CHROMOSOMAL	AUTOSOMAL
Human	DAZ cluster (at least 4 copies, > 99% identical	DAZL (chrom. 3)
Mouse		DAZL (Dazla) (chrom. 17)
Xenopus		Xdazl
Drosophila		boule
C. elegans		daz-1

Shortly after we began our Y chromosomal mapping study of infertile men, intracytoplasmic sperm injection (ICSI) with testicular and epididymal sperm retrieval methods for azoospermia were developed (Schoysman et al., 1993; Devroey et al., 1994; Silber et al., 1994, 1995a; Devroey et all, 1995a; Silber et al., 1995b, 1996; Silber 1998a; Silber et al., 1995; Tournaye et al., 1994; Mulhall et al., 1997). Men with the most severe spermatogenic defects causing azoospermia in the ejaculate could now have children. Thus, at the very moment in time that we had an effective treatment for severe male infertility, the reality that male infertility is often of genetic origin, also became generally recognized. Subsequently it was demonstrated that these Y deletions would be transmitted to offspring as a result of ICSI (Silber et al., 1998; Silber 1998a, 1998b; Page et al., 1999). When sperm were recoverable in azoospermic or oligospermic men, there was no significant difference in fertilization or pregnancy rate with ICSI whether the man was Y-deleted or not (Table 5.3 and 5.4). Large defects resulted in complete azoospermia but smaller defects were associated with the recovery of some sperm sufficient for ICSI, and even occasionally spontaneous offspring as well (Silber et al., 2001).

TABLE 5.3 RESULTS OF ICSI IN Y-DELETED VERSUS Y NON-DELETED MEN WITH SEVERE OLIGOSPERMIA (<2 X 10^6) AND AZOOSPERMIA (NON- OBSTRUCTIVE)

	Y-Deleted (<2 x 10^6)	Not Y-Deleted (<2 x 10^6)
N. Patients (with sperm)	23	205
N. Cycles (with sperm)	45	312
N.Eggs	508	3291
N. 2PN	(289) 57%	(1849) 56%
N. Pregnant	(17) 38%	(112) 36%
N. Deliveries	(13) 29%	(81) 26%
N. Babies	18	99
N. Boys	10	43
N. Girls	8	56

Silber, Oates, Brown, Page (2001)

TABLE 5.4 Y DELETION DETECTION IN PERIPHERAL LYMPHOCYTES OF 884 INFERTILE MEN (S/A <5X10^6)

	All Y Deletions	
Diagnosis	Number Studied	Y Deletion Found
Non-obstructive azoospermia	528	66(13%)
Severe oligospermia (<5x10^6)	356	24 (7%)
Totals	884	90 (10%)

As of December 2001

WHY THE "Y"?

Why should the initial molecular efforts at defining the genetic causes of male infertility have concentrated on this difficult Y chromosome with all of its confounding repeats, polymorphisms, and degenerating regions? The answer lies in the evolutionary history of the X and Y chromosome. Over the course of the last 240-320 million years of mammalian evolution, the X and

the Y chromosome have evolved from what was originally a pair of ordinary autosomes (Fig. 5.4) (Lahn and Page, 1997, 1999a; Rice 1992, 1994, 1996; Graves 1995a, 1995b, 2000). During that evolution, just as most of the ancestral X genes were decaying because of the lack of meiotic recombination of the developing X and Y chromosomes, genes which control spermatogenesis arrived (by transposition or retroposition) from autosomes to the Y (Fig. 5.5). Once on the Y, these formerly autosomal genes amplified into multiple copies, and achieved greater prominence (Saxena et al., 1996; Lahn and Page, 1999a). Spermatogenesis genes that arrived on the Y, but came originally from autosomes, include the well-studied DAZ and CDY (Saxena et al., 1996, 2000; Lahn and Page, 1999b) (Fig. 5.5). Other spermatogenesis genes on the Y have persisted from their original position on the X and developed specific spermatogenic function on the Y, and also into numerous copies on the Y, such as RBM (Delbridge et al., 1997; Vogel et al., 1999; Delbridge 1999a, 1999b; Mazeyrat et al., 1999).

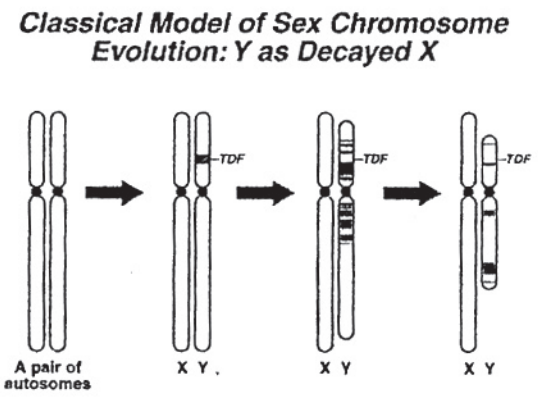


FIGURE 5.4. Figurative outline of evolutionary degeneration of one chromosome with the testicular determining factor (TDF) gene which doesn't recombine with its homologue, resulting eventually in a Y chromosome.

Although DAZ is a very ancient, well conserved gene, readily found to be functional in autosomes of c. elegas (worms), drosophila (fruit flies), xenopus (frogs), and rodents, it is only found on the Y chromosome of old world monkeys, apes, and humans (Table 5.2). In earlier mammals and in non-mammalian species, it is otherwise purely autosomal. RBM, however, is found on the mammalian Y as far back as the Y's origin, as evidenced by its presence on the Y of marsupials even before the divergence of eutherian from non-eutherian mammals. Thus, RBM was a spermatogenesis gene which

began on the ancestral autosomes that evolved into the mammalian X and Y chromosomes. The ancestral RBM that remained on the X chromosome (RBMX) retained its "widespread" function, whereas RBM-Y, which persisted on the receding Y chromosome, evolved a male-specific function in spermatogenesis (Delbridge et al., 1999; Mazeyrat et al., 1999; Graves, 1997; Pask et al., 1999).

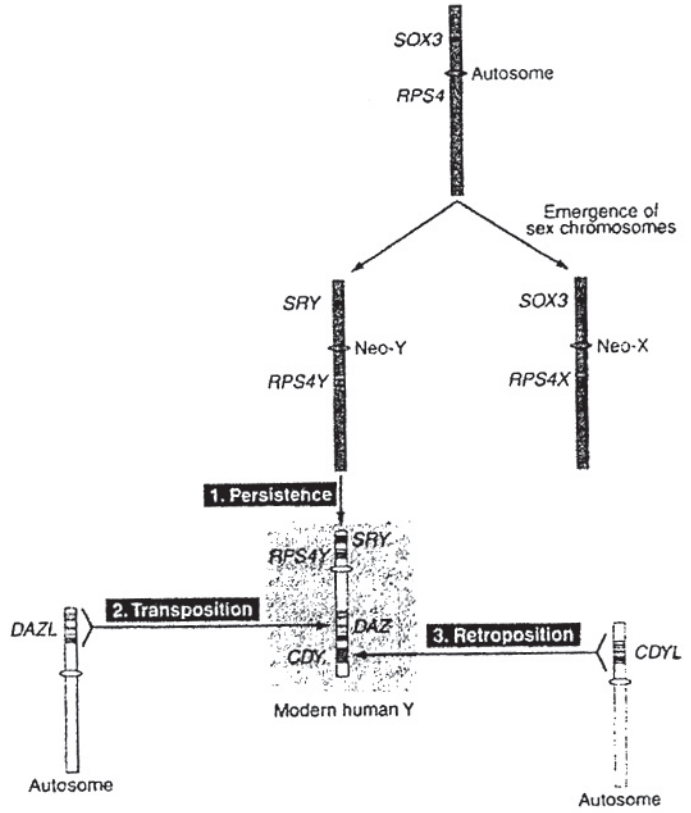


FIGURE 5.5. Over the course of evolution, the Y chromosome descended from the ancestral autosome that developed the SRY male-determining gene. The Y then attracted male-specific genes by three mechanisms (Lahn and Page modified, 1999b).

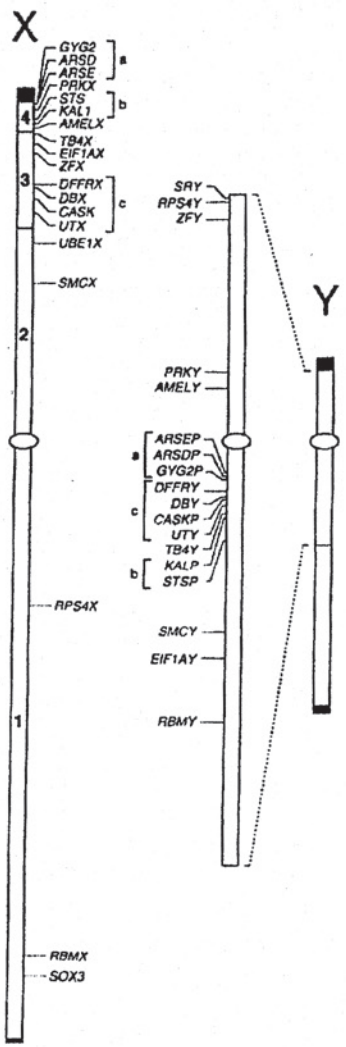


FIGURE 5.6. Graphic depiction of X-homologous genes on the Y chromosome representing four different stages of a divergence from its original ancestral X showing corresponding X and Y homologous genes. Note that SOX3 and RBMX come from the earliest region of sequence divergence and correlate with the SRY gene and the RBMY gene. (Lahn and Page, 1999a).

Indeed, even the SRY gene (the male sex-determining locus) was probably originally the SOX3 gene on the ancestral X prior to differentiating into the SRY male sex-determining gene. In fact, the evolution of a non-recombining male determining gene (SRY) is what actually began the whole process of the Y chromosome's evolution. SOX-3 is a gene on the X chromosome which inhibits SOX-9 also on the X chromosome. SOX-9 (on the X chromosome) is the gene that actually activates male sex determination. SOX-3 evolved into SRY on the ancestral Y chromosome. SRY inhibits SOX-3 from suppressing SOX-9, and thus determines whether the SOX-9 cascade of events leading to the formation of a testis takes place. That was the beginning of the transformation of an ordinary pair of autosomes into the modern X and Y. (Fig. 5.6) (Lahn and Page, 1999b; Graves 1997, 1995a, 1995b; Vidal et al., 2001).

Genes associated with the non-recombining SRY region that were specifically beneficial for male function or antagonistic to female function, flourished on the evolving Y chromosome because it was a "safe harbor," without the detrimental effect of meiotic recombination which would have otherwise allowed male-specific genes to be expressed in females (Lahn and Page, 1997, 1999a, 1999b; Silber 1999). In this way, "male benefit" genes have arrived and accumulated on the evolving Y chromosome over many millions of years via the three mechanisms of: "transposition" from an autosome via translocation, "retroposition" from an autosome via reverse transcription, and "persistence," i.e., male modification of function from what was originally a gene on the ancestral X. This process gives the Y chromosome a very unique type of "functional coherence" not seen elsewhere in the human genome (Fig. 5.7) (Lahn and Page, 1997). However, like with SRY, we should not be surprised to find that many genes which are male-specific could be on the X as well, and sprinkled throughout the genome.

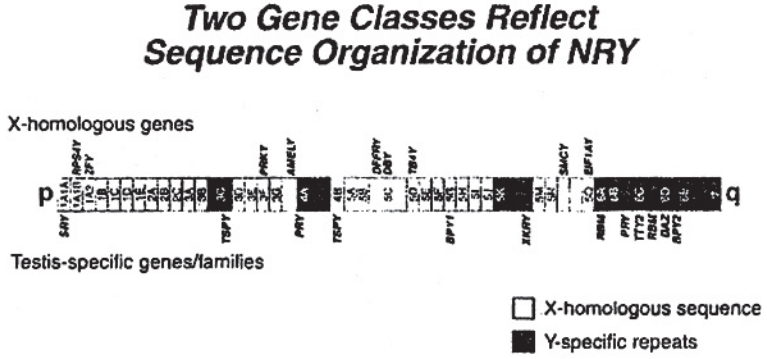


FIGURE 5.7. **Y chromosome has a remarkable functional coherence not seen in any other chromosomes. Genes depicted above are X-homologous because of their equal similarity to genes on the X chromosome. Genes depicted below are Y-specific genes which are testis specific, expressed only in the testis, and have no X-homologues. (Lahn and Page, modified, 1997).**

FUNCTIONAL REPRODUCTIVE ANATOMY OF THE X AND Y CHROMOSOME

Translocations occur on a relatively frequent basis in any species. Over evolutionary time, this results in conserved, homologous genes of different species residing in completely different parts of the genome and in a relatively mixed-up array of genes in every chromosome, where structural proximity has little or no relationship to function (Lahn and Page, 1997). However, these random transpositions (which over the course of time result in a chaotic lack of apparent organization of the genome) have also allowed direct acquisition by the Y of genes that have a common function to enhance male fertility. Selective pressures favor the process of spermatogenesis genes concentrating on the non-recombining portion of the Y chromosome in association with the male sex-determining gene, SRY, particularly if these genes are of little benefit to females or actually diminish "female fitness" (Saxena et al., 1996; Rice 1992, 1994, 1996; Silber 1999; Graves 1995; Winge 1927; Charlesworth and Charlesworth, 1980; Hackstein and Hochstenback, 1995).

Quite interestingly, the X chromosome, unlike autosomes, and unlike the Y chromosome, has been remarkably conserved in all mammals, with little mixing of genes from elsewhere. This is because of the selection against

disruption of development of the X-inactivation process in the evolution of the X and Y (Graves et al., 1998)

Genes which arrived on the Y, or which persisted on the degenerating Y from the ancestral X, and gained prominence on the Y, underwent paradoxical processes of amplification, producing multiple copies, and degeneration because of the failure of recombination. DAZ (as has been discussed) was the first such gene which was identified in the AZFc region of the Y chromosome by our initial Y mapping in azoospermic men (Reijo et al., 1995). DAZ represents the first unambiguous example of autosome-to-Y transposition of a spermatogenesis gene, which is representative of a generalized process that effects many other spermatogenesis genes, and indeed possibly explains the relatively poor state of affairs of human spermatogenesis compared to that of other animals (Saxena et al., 1996; Silber 1999). Autosome-to-Y transposition of male fertility genes appears to be a recurrent theme in Y chromosome evolution throughout all species. The autosomal DAZ gene (in humans called DAZ-L) is located on human chromosome 3, and on mouse chromosome 17. At some point during the evolution from new world to old world monkey, about 30 million years ago, this DAZ gene arrived on the Y by transposition from what is now human chromosome 3, and there multiplied to produce four almost identical gene copies. This process was first depicted for DAZL and DAZ. However, there are now known to be other previously autosomal genes or gene families on the Y that are expressed specifically in the testis, and are also likely to play a major role in spermatogenesis (Table 5.5) (Lahn and Page, 1997).

The CDY gene arrived on the AZFc region of the Y chromosome in a different fashion than DAZ, via reverse transcription (Lahn and Page, 1999a). The autosomal CDY gene (CDY-L) is located on mouse chromosome 13 and on human chromosome 6. CDY's intron-free homologue found its way to the human Y actually prior to the arrival of DAZ, sometime after the prosimian line of primates separated off, approximately 50 million years ago (Fig. 5.8). It did so by reverse transcription and, therefore, has very few introns in marked contrast to CDYL, its autosomal homologue on chromosome 6, which is intron-rich (Lahn and Page, 1999a).

TABLE 5.5 ARRIVAL OF SPERMATOGENESIS GENES TO Y CHROMOSOME

	Y Gene	Ancestral Gene
Transposition	DAZ (AZFc)	DAZL (autosomal)
	Human-Y	Mouse 17 Human 3
Retroposition	CDY (AZFc)	CDYL (autosomal)
	Human-Y	Mouse 13 Human 6
Persistence	RBM (AZFb)	RBMX
	Human-Y and Mouse-Y	Mouse X Human X
Persistence	SRY	SOX-3

The RBM gene, on the AZFb region of the Y chromosome, had its origin in our ancestral X chromosome, and there it amplified and gained prominence as a testis-specific gene. 240 to 320 million years ago, shortly after the divergence of the mammalian and avian lineages, the X and Y began to diverge in sequence identity with the emergence of SRY and the failure of recombination in the region of SRY (Fig. 5.9) (Lahn and Page, 1999b).

As the evolving Y chromosome underwent decay because of lack of recombination, these genes (which were originally X chromosomal) diverged in sequence on the Y, and those which had "male benefit" functions persisted (Table 5.6). The prime example of such genes, of course, is the SRY gene itself, that began as the generic SOX3 on the X chromosome, but then developed it's specialized "testis determining function," originating the whole process of the non-recombination, which resulted ultimately in degeneration of the Y chromosome (Lahn and Page, 1999b). This same process is how RBM arrived to prominence on the AZFb region of the Y (Lahn and Page, 1999b; Vogel et al., 1999; Delbridge et al., 1999a, 1999b; Mazeyrat et al., 1999; Graves 1995; Cooke et al., 1996).

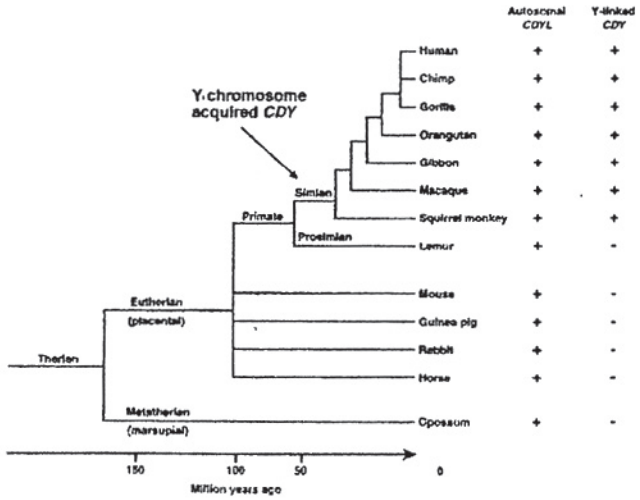


FIGURE 5.8. Whereas DAZ was transposed to the Y chromosome approximately 30 million years ago (after the divergence of old world and new world monkeys), CDY arrived on the Y chromosome much earlier (50 million years ago) by a process of reverse transcription. (Lahn and Page, 1999b).

The AZFa region of the NRY is a little more complicated. As the ancestral Y began to recede in comparison to its paired X, it did so in stages and "strata" over about 320 million years (Fig. 5.9). There are four clearly definable strata on the X chromosome that decrease in X-Y homology

according to how early in their history they failed to recombine (Lahn and Page, 1999a). As a given stratum of the X failed to recombine with its Y counterpart, homologous X-Y genes in that stratum diverged in sequence structure (Fig. 5.6). The most recent areas of non-recombination of X genes is located most proximally on the X and the most ancient areas of non-recombination are located most distally on the X. The AZFa region of the Y chromosome diverged from the X fairly recently in its evolutionary history and, therefore, has a much more conventional sequence structure, with much greater homology to its counterpart on the X. The two genes in AZFa (USP9Y and DBY) both play an important role in spermatogenesis, in that deletion of AZFa results in a complete absence of sperm. Yet they have very close homologues on the X, and are still ubiquitously transcribed (Sun et al., 1999, 2000).

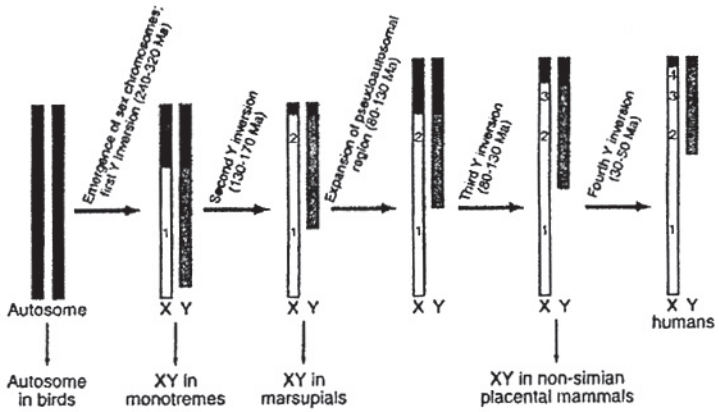


FIGURE 5.9. The X and the Y chromosome develop in mammals at the time of divergence in the avian and mammalian line by a series of four well defined inversions. The earliest inversions (region 1 on the X) have the least similarity to their Y-homologue, and genes in the most recent area of divergence (region 4) have the greatest sequence similarity of their counterpart on the Y. (Lahn and Page, modified, 1999a).

Regardless of the method of arrival of spermatogenesis genes to the non-recombining portion of the Y, this region would inevitably face, and likely succumb, to powerful degenerative forces during subsequent evolution (Saxena et al., 1996). Saxena postulated that, "perhaps the rate of acquisition of male fertility genes approximates the rate of subsequent degeneration, resulting in an evolutionary steady state. In contrast to the extreme evolutionary stability of the X chromosome, at least in mammals, individual

male infertility genes might not be long lived, in an evolutionary sense, on the Y chromosome."

Our emphasis on the Y chromosome for locating spermatogenesis genes to help in elucidating the causes of male infertility makes sense, because the Y has collected for us genes that otherwise would be hidden throughout the genome. However, it would be naïve to assume, in view of the evolutionary history of the X and the Y, that there are not equally powerful components for regulating spermatogenesis located also on the X chromosome and on the autosomes. Some have speculated that the instability of the Y chromosome may lead to an inexorable decline in sperm production in the evolution of any species, unless there is either sperm competition within the mating pattern of the species, or a method of continual recruitment of new spermatogenesis genes to the Y chromosome with subsequent amplification prior to ultimate degeneration (Silber 1999). The Y chromosome is a favorable place to begin a molecular search for genes that affect male fertility. But the very reason for starting with the Y emphasizes the likelihood of finding more such genes hiding throughout the genome.

TABLE 5.6 PERSISTENCE ON Y OF RBM X

Y Gene	**Ancestral Gene**
SRY	**SOX-3**
Determines Male Sex	No male specific function
RBM-Y	**RBM-X**
MALE SPECIFIC FUNCTION Numerous copies Many degenerate Same in all etherian mammals	*NO MALE SPECIFIC FUNCTION* One copy

PARALLEL AND INDEPENDENT EVOLUTION OF X AND Y CHROMOSOMES IN HUMANS AND ANIMAL MODELS: THE ORIGIN OF X-INACTIVATION (E.G., WORMS, FLIES, EVEN FISH)

Sex chromosomes have evolved independently many times in different genera with the same common theme. The chromosome with the sex-determining gene progressively loses the ability to recombine with its mate, accumulates mutations, and embarks on an inexorable deterioration. For example, the mammalian Y chromosome and the Drosophila Y chromosome (not to mention the ZW system in avians) have nothing in common with each other except their name, and the fact that they do not recombine with their larger counterpart, which is called the X chromosome. The X and Y

chromosomes evolved completely separately and differently in each of these well studied groups of species, but remarkably they evolved via the same common evolutionary theme.

If the Y chromosome of Drosophila has a deletion, the Drosophila is sterile. If the Y chromosome of the mouse or human has a deletion, the mouse or human is sterile. In any species thus far studied, if the Y chromosome has a significant deletion, that species is sterile. However, the genes that would have been deleted on the Drosophila Y, or the mouse Y chromosomes, are not the same genes that are deleted in the human Y. For example, the homologue of the human Y DAZ gene on Drosophila is autosomal, (the so-called "boule" gene), just as it is also autosomal in the mouse (DAZLA), and the deletion of this autosomal gene in Drosophila, or in the mouse, results in sterility just as readily as deletion of the Drosophila Y or the mouse Y chromosome (Cooke et al., 1996; Eberhart et al., 1996; Ruggiu et al., 1997). Deletion of the DAZ genes on the Y chromosome of humans often does not result in complete absence of spermatogenesis, possibly because the ancient DAZ autosomal homologue on chromosome 3, rescues spermatogenesis to some small extent. Deletion of AZFb genes, however, usually result in total absence of sperm, probably because there are no effective autosomal or X homologues to rescue spermatogenesis when these genes are deleted.

The same pattern is found in all species studied. The X and Y begin as a pair of ancestral autosomes in which a male-determining gene (which does not recombine with its homologue) begins the inexorable process of decay into what then becomes a Y chromosome. In some Drosophila, the Y chromosome has disappeared altogether, and the resultant XO male is sterile. Although the human Y chromosome (or for that matter, any of the mammalian Y chromosomes) has no nucleotide sequence similarity at all to the fruit fly's Y chromosome, the same mechanism of accumulation of spermatogenesis genes to a decaying male sex determining chromosome is operating (Silber, 1999). Thus, the Y chromosome of the Drosophila, and the mouse, is quite different than the Y chromosome of the human, but yet they appear to be the same because of the common two evolutionary themes in the development of the Y. One theme is its gradual decay from what was its autosomal homologue, but is now the X chromosome, and the second theme is its growth from acquisition and accumulation of male benefit specific genes from other parts of the genome.

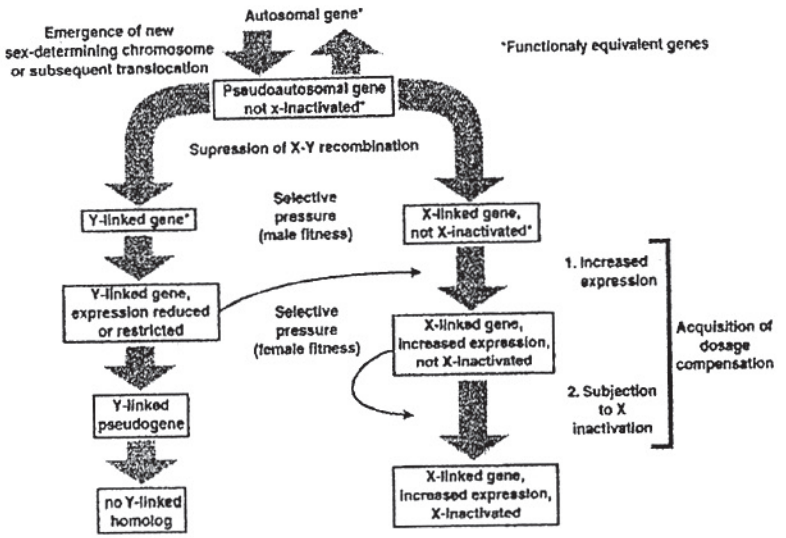
This evolutionary mechanism of degeneration of the Y, and accumulation of spermatogenesis genes may explain the relatively high frequency of male infertility and poor sperm quality in species (like ours) that have minimal sperm competition. It may also explain the phenomenon of X inactivation, the high frequency of XO human stillbirths, and the survival of some XO concepti as Turner's Syndrome children.

The ancestral autosome which is to become the X chromosome develops a process first of hyperactivation, and then X inactivation, to make up for the decay of homologous alleles on what is now becoming the Y chromosome (Jegalian and Page, 1998; Graves et al., 1998). As the X retained, and the Y gradually lost, most of these ancestral genes, expression of the X had to, at first, be increased to compensate for the male's loss of these genes, and X inactivation had to develop in the female for X genes whose Y homologue had eventually disappeared (Fig 5.10). The problem of X chromosome dosage differences in males and females is solved by inactivation in the female of one of the two X chromosomes combined with upregulation of the remaining X chromosome in females and the single X chromosome of males. This mechanism also insured the remarkable conservation and similarity of the X chromosome in all mammalian species.

An understanding of the evolution of X-homologous Y genes losing their general cellular functions, requiring upregulation, and inactivation of X genes on one of the two female X chromosomes, helps to clarify the different stages of evolutionary development of the mouse and human Y. The RBMY gene is a testis-specific male benefit spermatogenesis candidate gene. RBM's homologue on the X (RBMX) developed no male specific expression, but retained its general cellular housekeeping function. Thus, RBMX would be expected to behave like an X gene with no Y counterpart and probably undergo X inactivation (Table 5.7). As a related example, human ZFX and ZFY genes are very closely related homologues on the X and Y chromosome, both of which have general cellular housekeeping functions that are critical for life. Therefore, ZFX escapes X-inactivation in the female and ZFY is, therefore, probably one of the Turner genes (Lahn and Page, 1997; Jegalian and Page, 1998). However, the mouse is quite different. In the mouse, ZFY appears to have evolved a male-specific function, and, therefore, ZFX in the mouse has a general housekeeping function not shared with its Y homologue. Thus, ZFX in the mouse is X-inactivated, even though in the human it is not.

Similarly, RPS4X and RPS4Y are another homologous pair of genes on the X and Y, both of which have equivalent housekeeping functions in the human. Therefore, in the human RPS4X, similarly to ZFX, is not subject to X-inactivation because there are functional transcripts in men, from the X and Y, and in women from the two X's. In mice, however, RPS4Y has not only lost its function in evolution, but has degenerated out of existence. Therefore, in the mouse RPS4X is subject to X-inactivation, just as most of the *genes on the X chromosome in all animals require X-inactivation if they don't have a functioning homologue on the Y.*

This summary of the evolutionary history of our X and Y chromosome explains why the Y chromosome was a good place to start in our molecular search for spermatogenesis genes. However, it is clear that numerous genes from throughout the genome, though less well studied, also impinge on spermatogenesis, and may thus be transmitted to ICSI offspring.



Jegalian & Page, *Nature* 394:776 (1998)

FIGURE 5.10. X-inactivation develops after X-linked hyperexpression as a pairing mechanism between the evolving X and Y chromosomes to compensate for decay of X genes on the evolving Y. (Jegalian and Page, 1998).

KARYOTYPE OF INFERTILE MALES AND OF ICSI OFFSPRING

The incidence of cytogenetically recognizable chromosomal abnormalities in the offspring of ICSI patients is acceptably very low, but much greater than what would be anticipated in a normal newborn population. Follow-up of the first 1,987 children born as a result of ICSI has been meticulously studied and reported by the originators of ICSI in the Dutch-Speaking Free University of Brussels (Bonduelle et al., 1995, 1996, 1998a, 1998b, 1999). In 1,082 karyotypes of ICSI pregnancies, 9 (0.83%) had sex chromosomal abnormalities, including 45,X (Turners), 47, XXY (Klinefelter's), 47, XXX and mosaics of 47, XXX, as well as 47, XYY (Table 5.8). This is a very low frequency of sex chromosomal abnormalities, but nonetheless is four times greater than the expected frequency of sex chromosomal abnormalities in a newborn population (0.19%). Obviously the 45,X and 47,XXY children will be infertile (0.5%). Four (0.36%) of the 1,082 offspring had de novo balanced autosomal translocations or inversions. These children were apparently normal, but this incidence of de novo balanced autosomal translocations is

five times greater than what would be anticipated in a normal newborn population (0.07%), and these children might also be suspected of growing up to be infertile (0.36%).

TABLE 5.7 PERSISTENCE ON Y OF X GENES

	Y Gene	Ancestral X Gene
	SRY	SOX-3
	Determines male sex	No male specific function
	RPS4-Y	**RPS4-X**
Human	Housekeeping	Housekeeping
	Ubiquitous	Ubiquitous
	Turner Gene	No X-inactivation
Mouse	No RPS4-Y	Housekeeping
	Evolved out of existence	Is X-inactivated
	ZFY	**ZFX**
Human	Housekeeping	Housekeeping
	Ubiquitous	Ubiquitous
	Turner Gene	No X-inactivation
Mouse	Male specific function *only*	Housekeeping
	TWO COPIES ONLY	***UBIQUITOUS***
		Is X-inactivated

There were ten cases of translocations inherited from the infertile male (.92%), and these children are also likely to be infertile. Nine of these ten were balanced translocations in normal newborns. The one (0.09%) unbalanced translocation, was diagnosed at amniocentesis and was terminated. Since approximately 2% of oligospermic infertile males have chromosomal translocations (compared to a controlled population of 0.25%), it is not surprising that 0.9% of ICSI offspring would inherit such a translocation from their father (Van Assche et al., 1996). Thus, on purely conventional cytogenetic evidence, approximately 2% of ICSI offspring might be expected to share their father's infertility.

The remarkable five-fold increase in de novo balanced translocations among ICSI offspring (0.36% compared to 0.07%) is of great concern. Only 20% of balanced translocations are de novo, and 80% are inherited (Jaoobs et al., 1992). De novo balanced translocations are usually of paternal origin (84.4%) and obviously most of the inherited balanced translocations in ICSI patients would come from the father (Egozcue et al., 2000; Olson and Magenis, 1988). Balanced translocations which are associated with male infertility thus originally arose de novo in the testis of an otherwise fertile father, or his paternal ancestors, in 0.07% of a control population. Much more

frequently, de novo balanced translocations (albeit still a low percentage of only 0.36%) arise in the testis of infertile men undergoing ICSI and are transmitted to their offspring. The deficient testis appears not only to be at risk of transmitting inherited autosomal cytogenetic defects, but also of producing a greater number of de novo cytogenetic defects.

TABLE 5.8 KARYOTYPE ANOMALIES IN 1,082 PRENATAL DIAGNOSIS

Abnormal Karyotypes On 1,082 Prenatal Tests	Maternal Age (years)	Number	Percent	Percent in Literature
De novo chromosomal aberrations		18	1.66	*0.445*
Sex-chromosomal:		9	0.83	0.19, *0.23*
45, X	37			
46, XX/47, XXX	44			
47, XXX (2 children)	32, 37			
47, XXY (4 children)	26, 28, 28, 32			
47, XYY	25			
Autosomal:		9f	0.83	0.21, 0.61
Trisomy 21 (5 children)	32. 33, 37, 41, 41	5	0.46	0.14
structural		4	0.36	0.07
46, XXY, t (4;5)	30x			
46, XX, t (2;15)	30			
46, XX, t (2;13)	36			
46, XX, inv (1qh)	39			
Inherited aberrations		10	0.92	0.47
balanced		9	0.83	0.45
unbalanced		1	0.09	0.023
Total aberrations de novo + inherited		28	2.5	0.92, 0.84

(Bonduelle et al., 1995; Bonduelle et al., 1996; Bonduelle et al., 1998; Bonduelle et al., 1999)

The incidence of congenital abnormality in ICSI children (2.3%) is no greater than in every normal population studied (Bonduelle et al., 1995, 1996, 1998a, 1998b, 1999). Even the few reported ICSI offspring of Klinefelter's patients have been chromosomally normal (Palermo et al., 1998; Tournaye et al, 1996; Staessen et al., 1996; Levron et al., 2000). There is no greater incidence of autosomal aneuploidy than what is predictable from maternal age. Sex chromosome aneuploidy (0.83%) in ICSI offspring is not an unacceptably high incidence, although it is clearly greater than normal (0.19%). Thus, the evidence based on cytogenetic and pediatric follow-up of ICSI offspring is very reassuring, despite the probable occurrence of infertility and sex chromosomal disorders in a small percentage of cases. Study of the Y

chromosome, however, leads to even greater concern regarding the future fertility of these children.

Y DELETION STUDIES OF ICSI OFFSPRING

Microdeletions on the long arm of the Y chromosome do not appear to adversely effect the fertilization or pregnancy results either in severely oligospermic men, or in azoospermic men from whom sperm were successfully retrieved (Silber et al., 1998). There have been concerns registered that the ICSI results might be poorer with Y-deleted men, but in larger series, that has not been the experience (Van Golde et al., 2001; Silber, 2001) (Table 5.3).

Thus far, all of our male offspring from Y-deleted men have had the same Y deletion as their infertile father (Page et al., 1999). Fathers, brothers, and paternal uncles of the infertile men, were also examined for Y deletions and fertility. Y deletions in our infertile males were de novo for the most part. That is, the fertile fathers of the infertile Y-deleted patients had no Y deletion. However, all male offspring from ICSI procedures involving these Y-deleted men had their father's Y deletion transmitted to them without amplification or change (Fig. 5.11).

The idea that the Y deletion would be transmitted to the son is not as obvious as it might at first seem. If a few foci of spermatogenesis in the testis of a severely oligospermic or azoospermic Y-deleted man were present because of testicular mosaicism, it would seem very possible that the few areas of normal spermatogenesis within such a deficient testis of a Y-deleted man might actually have a normal Y chromosome. In that event, one could have expected the sons of these patients undergoing ICSI not to be Y-deleted. For example, thus far all the sons of Klinefelter's patients have been normal 46, XY (Palermo et al., 1998; Tournaye et al., 1996; Staessen et al., 1996; Levron et al., 2000). Thus, it is not at all obvious, intuitively, that this Y deletion had to be transmitted to the son. However, increasing experience seems to indicate that the Y deletion of the sterile father is, in fact, transmitted to the son, and we no longer have to just speculate about it.

It remains to be determined whether non Y-deleted fertile or infertile men have mosaic deletions in their testis. If so, then de novo Y deletions would also be found more frequently in the brothers of our Y-deleted patients, or in ICSI offspring of infertile men (even those who have no Y deletion) than would otherwise be expected to occur in a normal newborn population (Kent-First et al., 1996). However, what we now know from the detailed sequence studies of the AZFa and AZFc regions of the Y chromosome gives us a much better picture of how Y deletions commonly occur, and how they are transmitted to offspring.

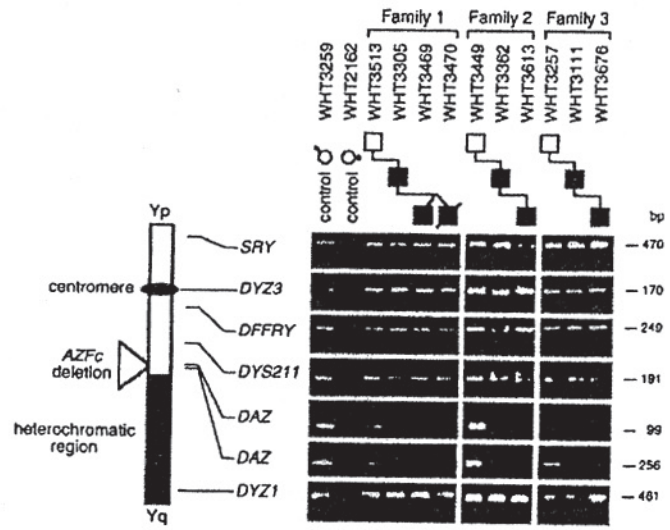


FIGURE 5.11. The AZFc Y deletion present in the azoospermic or severely oligospermic father is not present in his father, but is transmitted to his sons via ICSI. (Page, Silber, Brown, 1999).

MECHANISM OF DE NOVO APPEARANCE OF Y DELETION, AND ITS TRANSMISSION TO FUTURE GENERATIONS

The first region of the Y chromosome that was completely sequenced was AZFa because it was a region of the Y with very little repetitive sequences, and relatively amenable to study. Now, the more daunting AZFc region (with large areas of sequence identity) has also been very recently sequenced (Kuroda-Kawaguchi et al., 2001). The sequence of AZFa, revealed it to span approximately 800,000 nucleotide bases (800 KB), and was bounded on each side by a proximal breakpoint area and a distal breakpoint of around 10,000 bases (10 KB) of 94% sequence identity with each other. Furthermore, the sites of these breakpoints (even with conventional mapping) in most infertile men with AZFa deletions were indistinguishable from each other. Within these 10 KB breakpoint regions, the site of AZFa deletion almost uniformly fell within smaller domains (447 BP to 1285 BP) of these 10,000KB breakpoints that exhibited *absolute sequence* identity (Sun et al., 2000). Indeed, the sequencing of deletion junctions of most AZFa-deleted patients revealed that homologous ("illegitimate") recombination had occurred between identical areas of proviruses that bounded each side of this 800,000 base region, allowing the entire intervening segment to drop out. The repeat

areas of absolute sequence identity proximal and distal to a common area of deletion gives us a clue to the mechanisms of these Y deletions. With AZFa, the sequence repeats are caused by an ancient intrusion of a retrovirus into that region of the human Y. For AZFc, the situation is similar but occurs for a different reason on a vast scale of unprecidented and much more massive lengths of repeats.

The findings in AZFa give a clue to what is operating in the more common areas of deletion, such as AZFc. The sequenc of AZFc reveals the same mechanism as for AZFa but on a grander scale. Large domains of absolute sequence identity become easy sites for drop-out of large chunks of DNA, as the boundaries of absolute sequence identity illegitimately recombine with each other (Tilford et al., 2001; Kuroda-Kawaguchi et al., 2001). Because of amplification and inversions in the most ancient areas of divergence of the non-recombining Y, the whole situation is a set-up for deletion and degeneration. The very repetitive nature of the Y chromosome, that made sequencing and finding small deletions or point mutations so difficult, is the cause of its instability both over an evolutionary time frame, as well as in our current infertile patient population. Y deletions large enough to be detected with our outmoded maps occur in about 1/2000 births because of these vast areas of absolute sequence identity.

The AZFc region of the Y, which is the most common deletion site, spans 3.5 megabases of DNA, and is thus hardly a "microdeletion" (as it is often called). It is composed of three giant palindromes constructed from six families of cmplicons (i.e., long areas of absolute sequence identity). It contains 19 transcriptional units composed of seven different gene families, only one of which is DAZ. The 3.5 mB AZFc region is bounded on each side by 229,000 long areas of near absolute sequence identity (99.9%). Unlike AZFa, the "breakpoints" of sequence identity did not come from an ancient retrovirus, but rather from the very nature of the evolution of the Y chromosome because of the failure of recombination. There are multiple other areas on the Y (and in AZFc) that have either direct or inverted sequence repeats. Direct repeat sequences will result in common deletions due to homologous recombination. Inverted repeats will result in "isodicentric" translocations also because of homologous recombination. Thus, we can expect to find many other, smaller deletions that have previously escaped detection by crude, non-sequence based STS mapping.

It may very well be that smaller deletions, taking out less genes, or point mutations in just one or two copies of identical genes that occur in multiple copies, could account for many more cases of male infertility, or perhaps more moderate degrees of spermatogenic failure (e.g. > $2x10^6$ to 20x10 sperm/cc). The large "micro-deletions" thus far reported in the literature are for the most part de novo, but certainly some men with severe oligospermia can naturally father children (about 5%). Men with more moderate degrees of oligospermia may father children even more easily, and thus smaller Y

deletions causing male infertility may indeed not as often be de novo. In any event, as more genetic causes of spermatogenic failure (severe or mild) come to light, there will be an increased awareness of the possible transmission of this infertility to future generations.

THE X CHROMOSOME AND MALE INFERTILITY

It has been theorized that the Y is not the only sex chromosome that accumulates genes which benefit spermatogenesis over an evolutionary time span (Rice 1984, 1992; Brooks 2000; Fisher 1931). As the X chromosome (240 to 320 million years ago) differentiated from the Y, the sexually antagonistic gene theory favors the emergence of genes on the X also that benefit the heterogametic sex (with mammals of course, that is the XY male) and are detrimental to the homogametic sex (the XX female) *if these genes are recessive*. For example, a rare recessive evolutionary mutation on the X that favors spermatogenesis would be preferentially passed on to male offspring who by virtue of a higher sperm count would then continue to pass down this favorable X mutation to his offspring. Such a recessive mutation (favorable to spermatogenesis) in an autosome would be lost to future generations. Thus, we can also anticipate an accumulation on the X chromosome (as well as the Y) of male benefit recessive genes.

In fact, RT-PCR subtraction studies of spermatogonia in mice have demonstrated that a large fraction of genes which are expressed exclusively in pre-meiotic male germ cells, are indeed X chromosomal in origin (Wang et al., 2001). Eleven of the 36 genes that were expressed specifically in mouse spermatogonia were found exclusively on the X chromosome. Since the X chromosome is so well conserved in all mammals (as explained earlier by the universal development of X inactivation in mammalian evolution), it seems very likely that evolution has also conferred on the human X chromosome a large portion of the burden for spermatogenesis. Thus, a search for detrimental mutations on the human X in infertile males is also likely to be very rewarding. Thus, the failure to identify a Y deletion gives no assurance whatsoever that a genetic cause for infertility won't be transmitted to the ICSI offspring either via the X, or even autosomes.

CONCLUSIONS

The presence of Y deletions does not decrease the fertilization or pregnancy rate for azoospermic and severely oligospermic (<$2x10^6$) men. Thus far the sex ratio of delivered children is apparently equal and the children are karyotypically normal. However, the Y deletion (and presumably infertility) is transmitted to the male offspring (Page et al., 1999). Although using standard STS mapping, Y deletions occur in only 10% of azoospermic

and severely oligospermic men, sequenced based mapping (now just available) may increase that percentage significantly.

There are most likely many spermatogenesis genes involved in male infertility, and we have barely scratched the surface with what have been, up till now, very crude mapping techniques on the Y chromosome. Whether or not these gross "microdeletions" currently reported in the literature are found in an infertile male patient, does not obviate the likelihood of there being a genetic cause for his azoospermia or severe oligospermia. If a defective gene (or genes) is located on his Y chromosome, then his male offspring will most likely inherit his problem. However, there are also many genes on the X chromosome, and throughout the genome, that impinge upon spermatogenesis that are not thus far identified by our currently crude mapping procedures. The recognized failure of any conventional therapy to improve spermatogenesis infers a genetic origin for most spermatogenic defects (Silber 2000a; Silber 2000b; Silber 2001). These numerous genes may also be responsible for many cases of male infertility. Therefore, sons, and even daughters, may inherit the defect or be carriers.

It is clear that a negative Y microdeletion assay by currently popular methods does not rule out genetic abnormality. Therefore, in our view, genetic counseling should be provided to all infertile males, whether or not an abnormality is detected and whether or not Y deletion assays have even bothered to be performed. Although karyotyping certainly should be routinely performed for infertility patients (because of the risk of miscarriage and abnormal offspring resulting from either sex chromosome abnormalities, or unbalanced translocations), Y deletion testing may not be mandatory yet, because it is still very crude, and negative results should not be at all reassuring. Furthermore, some "deletions" may only be polymorphisms, and not of clinical significance. It is apparent that there is likely to be frequent transmission of male infertility from the ICSI father to his male (or even female) offspring regardless of current testing. Every couple must decide for themselves whether they wish to consider this risk. In our experience, most such couples, even when well informed, choose to have ICSI despite this risk. Thus, continued long-term clinical and molecular study of ICSI offspring is mandatory.

REFERENCES

Anguiano, A., Oates, R.., Amos, J. et al.. (1992). Congenital bilateral absence of the vas deferens: a primarily genital form of cystic fibrosis. JAMA **267**: 1794-1797.

Baker, H., Burger, H., de Kretser, D et al. (1981). Factors affecting the variability of semen analysis results in infertile men. Int. J. Androl. **4**: 609-622.

Baker, H.., Straffon, W.., McGowan, M., et al., (1984). A controlled trial of the use of erythromycin for men with asthenospermia. Intl. J. Androl. **7**: 383-388.

Baker, H., Burger H., de Kretser, D. et al., (1985). Testicular vein ligation and fertility in men with varicoceles. British Med. J. **291**: 1678-1680.

Baker, H. and Kovacs, G. (1985). Spontaneous improvement in semen quality: regression towards the mean. Intl. J. Androl. **8**: 421-426.

Baker, H.. (1986). Requirements for controlled therapeutic trials in male infertility. Clin. Reprod Fertil **4**: 13-25.

Bhasin, S., deKretser, D.M., and Baker, H.W.G. (1994). Pathophysiology and natural history of male infertility. J Clin. Endocrinol. Metab. **79**: 1525-1529.

Bonduelle, M., Legein, J., Derde, M., et al. (1995). Comparative follow-up study of 130 children born after ICSI and 130 children after IVF. Human Reprod **10**: 3327-3331.

Bonduelle, M., Wilikens, J., Buysse, A., et al. (1996). Prospective study of 877 children born after intracytoplasmic sperm injection with ejaculated, epididymal, and testicular spermatozoa, and after replaced of cryopreserved embryos obtained after ICSI. Hum Reprod **11**: 131-159.

Bonduelle, M., Aytoz, A., Wilikens, A., et al. (1998a). Prospective follow-up study of 1,987 children born after intracytoplasmic sperm injection (ICSI). In: Filicori M, Flamigni C, eds. **Treatment of Infertility: The New Frontiers**. Princeton: Communications Media for Education, 445-461.

Bonduelle, M., Aytoz, A., Van Assche, E., et al., (1998b). Incidence of chromosomal aberrations in children born after assisted reproduction through intracytoplasmic sperm injection. Hum Reprod **13**: 781-782.

Bonduelle, M., Camus, M., De Vos, A., et al. (1999). Seven years of intracytoplasmic sperm injection and follow-up of 1,987 subsequent children. Hum Reprod **14**: 243-264.

Brandell, R., Mielnik, A., Liotta, D., et al., (1998). AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. Hum Reprod **13**: 2812-2815.

Brooks, R. (2000) Negative genetic correlation between male sexual attractiveness and survival. Nature **406**: 67-70.

Chai, N., Salido, E., and Yen, P. (1997). Multiple functional copies of the RBM gene family, a spermatogenesis candidate on the human Y chromosome. Genomics **45**: 355-361.

Chai, N., Zhou, H., Hernandez, J.,et al. (1998). Structure and organization of the RBMY genes on the human Y chromosome: transposition and amplification of an ancestral autosomal hnRNPG gene. Genomics **49**: 283-289.

Chang, P., Sauer, M., and Brown, S. (1999). Y chromosome microdeletion in a father and his four infertile sons. Hum. Reprod **14**: 2689-2694.

Charlesworth, D., and Charlesworth, B. (1980). Sex-differences in fitness andselection for centric fusions between sex chromosomes and autosomes. Genet. Res. **35**: 205-214.

Chillón, M., Casals, T., Mercier, B, et al., (1995). Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N. Eng.l J. Med. **332**: 1475-1480.

Clermont, Y. (1972). Kinetics of spermatogenesis in mammals: Seminiferous epithelium cycles in spermatogonial renewal. Physiol. Rev. **52**: 198-236.

Cooke, H., Lee, M., Kerr, S. and Ruggiu, M. (1996). A murine homologue of the human DAZ gene is autosomal and expressed only in male and female gonads. Hum. Mol. Genet. **5**: 513-516.

Cram, D., Ma, K.. Bhasin, S., et al. (2000). Y chromosome analysis of infertile men and their sons conceived through intracytoplasmic sperm injection: vertical transmission of deletions and rarity of de novo deletions. Fertil Steril **74:** 909-915.

Delbridge, M., Harry, J., Toder, R., et al. (1997). A human candidate spermatogenesis gene, RBM1, is conserved and amplified on the marsupial Y chromosome. Nature Genetics **15:** 131-136.

Delbridge, M. and Graves, J. (1999). Mammalian Y chromosome evolution and the male-specific functions of Y chromosome-born genes. Rev. Reprod **4:** 101-109.

Delbridge, M., Lingenfelter, P., Disteche, C. and Graves, J. (1999). The candidate spermatogenesis gene RBMY has a homologue on the human X chromosome. Nature Genetics **22:** 223-224.

deVries, J., Repping, S., Oates,R., et al., (2001). Absence of deleted in azoospermia (DAZ) genes in spermatozoa of infertile men with somatic DAZ deletions. Fertil Steril **75:** 476-479.

deVries, J., Hoffer, J., Repping, S. et al., . (2002). Reduced copy number of the DAZ genes in sub- and infertile men. Fertil Steril **77:** 68-75.

Devroey, P., Liu, J., Nagy, A., (1994). Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection (TESE and ICSI). Fertil Steril, **62:** 639-641.

Devroey, P., Silber, S., Nagy, Z., et al. (1995a). Ongoing pregnancies and birth after intracytoplasmic sperm injection (ICSI) with frozen-thawed epididymal spermatozoa. Hum Reprod **10:** 903-906.

Devroey, P., Liu, J., Nagy, Z., et al. (1995b). Pregnancies after testicular extraction (TESE) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia. Hum. Reprod **10:** 1457-1460.

Devroey, P., Vandervorst, M., Nagy, P., and Van Steirteghem , A. (1998). Do we treat the male or his gamete? Hum. Reprod **13:** 178-185.

Eberhart, C., Maines, J. and Wasserman, S.. (1996). Meiotic cell cycle requirement for a fly homologue of human Deleted in Azoospermia. Nature **381:** 783-785.

Edwards, R. and Bishop, C. (1997). On the origin and frequency of Y chromosome deletions responsible for severe male infertility. Mol. Hum. Reprod **3:** 549-554.

Egozcue, S., Blanco, J., Vendrell, J., et al. (2000). Human male infertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. Hum. Reprod Update **6:** 93-105.

Elliott, D., Millar, M., Oghene, K, et al. (1997). Expression of RBM in the nuclei of human germ cells is dependent on a critical region of the Y chromosome long arm. Proc. Natl. Acad. Sci. USA **94:** 3848-3853.

Faddy, M., Silber, S. and Gosden, R. (2001). Intra-cytoplasmic sperm injection and in fertility. Nature Genetics **29:** 1310.

Fisher, R.. (1931). The evolution of dominance. Biol. Rev. **6:** 345-368.

Foote, S., Vollrath, D., Hilton, A. and Page, D. (1992). The human Y chromosome: Overlapping DNA clones spanning the euchromatic region. Science **258:** 60-66.

Foresta, C., Ferlin, A., Garolla, A., et al. (1997). Y-chromosome deletions in idiopathic severe testiculopathies. J. Clin. Endocrino.l Metab. **82:** 1075-1080.

Foresta, C., Ferlin, A., Garolla, A., et al. (1998). High frequency of well-defined Y-chromosomal deletions in idiopathic Sertoli cell-only syndrome. Hum. Reprod **13:** 302-307.

Girardi, S., Mielnik, A., and Schlegel, P. (1997). Submicroscopic deletions in the Y chromosome of infertile men. Hum. Reprod **12:** 1635-1641.

Graves, J. (1995a). The origin and function of the mammalian Y chromosome and Y- borne genes – an evolving understanding. Bioessays **17:** 311-320.

Graves, J.. (1995b). The evolution of mammalian sex chromosomes and the origin of sex determining genes. Phi.l Trans. R. Soc. Lond. B **350:**305-312.

Graves, J. (1997). Two uses for old SOX. Nature Genetics **16:** 114-115.

Graves, J. (2000). Human Y chromosome, sex determination, and spermatogenesis - a feminist view. Bio.l Reprod **63:** 667-676.

Graves, J., Disteche, C., and Toder, R. (1998). Gene dosage in the evolution and function of mammalian sex chromosomes. Cytogenet. Cell Genet. **80:** 94-103.

Grimaldi, P., Scarponi, C., Rossi, P. et al. (1998). Analysis of Yq microdeletions in infertile males by PCR and DNA hybridization techniques. Mol. Hum. Reprod **4:** 1116-1124.

Hackstein, J. and Hochstenback, R. (1995). The elusive fertility genes of Drosophila: the ultimate haven for selfish genetic elements. Trends Genet. **11:** 195-200.

Jacobs, P., Broune, C., Gregson, N., et al., (1992). Estimates of the frequency of chromosome anomalies detectable using moderate levels of banding. J. Med. Genet. **29:** 103-108.

Jegalian, K. and Page, D. (1998). A proposed path by which genes common to mammalian X and Y chromosomes evolve to become X inactivated. Nature **394:** 776-780.

Jegalian, K. and Lahn, B.. (2001). Why the Y. Sci. Am. 56-61.

Johnson, M., Tho, S., Behzadian, A. and McDonough, P. (1989). Molecular scanning of Yq11 (interval 6) men with Sertoli-cell-only syndrome. Am. J. Obstet. Gynecol. **161:** 1732-1737.

Kent-First, M., Kol, S., Muallem, A., et al. (1996). The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. Mol. Hum. Reprod **2:** 943-950.

Kent-First, M., Muallem, A., Shultz, J. et al. (1999). Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. Mol. Reprod Dev. **53:** 27-41.

Kim, S., Kim, K. and Paick, J. (1999). Microdeletions within the azoospermia factor subregions of the Y chromosome in patients with idiopathic azoospermia. Fertil Steril **72:** 349-353.

Krausz, C., Bussani-Mastellone, C., Grauchi, S. et al. (1999). Screening for microdeletions of the Y chromosome genes in patients undergoing intracytoplasmic sperm injection. Hum. Reprod **14:** 1717-1721.

Krausz, C. and McElreavey, K. (2001) Y chromosome microdeletions in 'fertile' males. Hum. Reprod (letter) **16:** 1306-1307.

Kremer, J., Tuerlings, J., Meuleman, E.., et al. (1997) Microdeletions of the Y chromosome and intracytoplasmic sperm injection: from gene to clinic. Hum. Reprod **12:** 687-691.

Kremer, J., Tuerlings, J., Borm, G., et al. (1998) Does intracytoplasmic sperm injection lead to a rise in the frequency of microdeletions in the AZFc region of the Y chromosome in future generations? Hum. Reprod **13:** 2808-2811.

Kuroda-Kawaguchi, T., Skaletsky, H., Brown, L., et al., (2001) The human Y chromosome's AZFc region features massive palindromes, uniform recurrent deletions, and testis gene families. Nature Genetics **29:** 279-286.

Lahn, B. and Page, D. (1997) Functional coherence of the human Y chromosome. Science, **278:**675-680.

Lahn, B. and Page, D. (1999a) Four evolutionary strata on the human X chromosome. Science **286:** 964-967.

Lahn, B. and Page, D. (1999b) Retroposition of autosomal in RNA yield testis-specific gene family on human Y chromosome. Nature Genetics **2:** 429-433.

Lahn, B. and Page, D. (2000) A human sex-chromosomal gene family expressed in male germ cells and encoding variably charged proteins. Mol. Hum. Genet. **9:** 311-319.

Levron, J., Aviram-Goldring, L., Madgar, I., et al., (2000) Sperm chromosome analysis and outcome of IVF in patients with non-mosaic Klinefelter's Syndrome. Fertil Steril **74:** 925-929.

Liow, S., Ghadessy, F., N, S. and Yong, E. (1998). Y chromosome microdeletions in azoospermic or near-azoospermic subjects are located in the AZFc (DAZ) subregion. Mol. Hum. Reprod. **4:** 763-768.

Liu, J., Nagy, Z., Joris, H., et al., (1994). Intracytoplasmic sperm injection does not require a special treatment of the spermatozoa. Hum Reprod **9:** 1127-1130.

Liu, J., Nagy, Z., Joris, H.,et al. (1995). Analysis of 76 total fertilization failure cycles out of 2,732 intracytoplasmic injection cycles. Hum. Reprod **10:** 2630-2636.

Ma, K., Inglis, J., Sharkey, A., et al. (1993). A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. Cell **75**: 1287-1295.

Ma, K., Sharkey, A., Kirsch, S., et al., (1992). Towards the molecular localisation of the AZF locus: mapping of microdeletions in azoospermic men within 14 subintervals of interval 6 of the human Y chromosome. Hum. Mo.l Genet. **1**: 29-33.

Mazeyrat, S., Saut, N., Mattei, M. and Mitchell, M. (1999). RBMY evolved on the Y chromosome from a ubiquitously transcribed X-Y identical gene. Nature Genetics **22**: 224-226.

Menke, D., Mutter, G. and Page, D. (1997). Expression of DAZ, an azoospermia factor candidate, in human spermatogonia. Am. J. Hum. Genet. **60**: 237-241.

Morris, R. and Gleicher, N. (1996). Genetic abnormalities, male infertility, and ICSI. Lancet **347**: 1277.

Mulhall, J., Reijo, R., Alagappan, R., et al. (1997). Azoospermic men with deletion of the DAZ gene cluster are capable of completing spermatogenesis: Fertilization, normal embryonic development and pregnancy occur when retrieved testicular spermatozoa are used for intracytoplasmic sperm injection. Hum. Reprod **12**: 503-508.

Najmabadi, H., Huang, V., Yen, P., et al. (1996). Substantial prevalence of microdeletions of the Y-chromosome in infertile men with idiopathic azoospermia and oligozoospermia detected using a sequence-tagged site-based mapping strategy. J. Clin. Endo. Metab. **81**: 1347-1352.

Nagy, Z., Liu, J., Joris, H., et al., (1995). The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters. Hum. Reprod **10**: 1123-1129.

Nakahori, Y., Kuroki, Y., Komaki, R., et al. (1996). The Y chromosome region essential for spermatogenesis. Hormone Res. **46**: 20-23.

Nieschlag, E., Hertle, L., Fischedick, A. and Behre, H. (1995). Treatment of varicocele: counselling as effective as occlusion of the vena spermatica. Hum Reprod **10**: 347-353.

Nieschlag, E., Hertle, L., Fischedick, A., et al., (1998). Update on treatment of varicocele: counselling as effective as occlusion of the vena spermatica, Hum. Reprod **13**: 2147-2150.

Nilsson, S., Edvinsson, A. and Nilsson, B. (1979). Improvement of semen and pregnancy rate after ligation and division of the internal spermatic vein: Fact or fiction? British J. Urol. **51**: 591-596.

O'Brien, S., Wildt, D. and Bush, M. (1986). The cheetah in genetic peril. Sci. Am. **254**, 84-92.

O'Brien, S., Wildt, D., Bush, M., et al. (1987). East African cheetcas: evidence for two population bottlenecks? Proc. Nat.l Acdd. Sci. USA **84**: 508-511.

Oliva, R., Margarit, E., Ballesca, J-L. et al. (1998). Prevalence of Y chromosome microdeletions in oligospermic and azoospermic candidates for intracytoplasmic sperm injection. Fertil Steril **70**: 506-510.

Olson, S. and Magenis, R. (1988). Preferential paternal origin of de novo structural rearrangements. In: Daniel A, ed. **The Cytogenetics of Mammalian Autosomal Rearrangements**. New York: Alan R. Liss, 583-599.

Page, D., Silber, S. and Brown, L. (1999). Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. Hum. Reprod **14**: 1722-1726.

Palermo, G., Joris, H., Devroey, P. et al. (1992). Pregnancies after intracytoplasmic injection of a single spermatogoa into an oocyte. Lancet **3**: 17-18.

Palermo, G., Schlegel, P., Sills, E., et al. (1998). Births after intracytoplasmic injection of sperm obtained by testicular extraction from men with nonmosaic Klinefelter's syndrome. N. Engl. J. Med. **338**: 588-590.

Pask, A. and Graves, J. (1999). Sex chromosomes and sex-determining genes: insights from marsupials and monotremes. Cell Mol. Life Scr. **55**: 864-875.

Prosser, J., Inglis, J., Condie, A., et al.,(1996). Degeneracy in human multicopy RBM (YRRM), a candidate spermatogenesis gene. Mamm. Genome **7**: 835-842.

Pryor, J., Kent-First, M., Muallem, A., et al. (1997). Microdeletions in the Y chromosome of in fertile men. N. Eng. J. Med. **336**: 534-539.

Qureshi, S., Ross, A., Ma, K.,et al. (1996). Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically-determined spermatogenic failure in men. Mol. Hum. Reprod **2**: 775-779.

Reijo, R., Lee, T., Salo, P., et al, (1995). Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nature Genetics, **10**: 383-393.

Reijo, R., Alagappan, R. Patrizio, P. and Page, D. (1996). Severe oligozospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet **347**: 1290-1293.

Rice, W. (1984). Sex-chromosomes and the evolution of sexual dimorphism. Evolution **38**: 735-742.

Rice, W. (1992). Sexually antagonistic genes: experimental evidence. Science **256**: 1436-1439.

Rice, W. (1994). Degeneration of a non-recombining chromosome. Science **263**: 230-232.

Rice, W. (1996). Evolution of the Y sex chromosome in animals. Bio. Science **46**: 331-343.

Rodriguez-Rigau, L., Smith, K. and Steinberger, E. (1978). Relationship of varicocele to sperm output and fertility of male partners in infertile couples. J. Urol. **120**: 691-694.

Ruggiu, M., Speed, R., Taggart, M., et al. (1997). The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. Nature **389**: 73-77.

Saxena, R., Brown, L., Hawkins, T., et al. (1996). The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. Nature Genetics **14**: 292-299.

Saxena, R., deVries, J., Repping, S., et al. (2000). Four DAZ genes in two clusters found in the AZFc region on the human Y chromosome. Genomics **67**: 256-267.

Schoysman, R. (1983). Twelve-year follow-up study of pregnancy rates in 1291 couples with idiopathically impathically impaired male fertility. Acta Europaea Fertilitatis **14**: 51-56.

Schoysman, R., Vanderzwalmen, P., Nijs, M., et al. (1993). Pregnancy after fertilization with human testicular spermatozoa. Lancet **342**: 1237.

Seifer, I., Amat, S., Delgado-Viscogliosi, P. et al. (1999). Screening for microdeletions on the long arm of chromosome Y in 53 infertile men. Int. J. Androl. **22**: 148-154.

Shin, D., Gilbert, F., Goldstein, M. and Schlegel, P. (1997). Congenital absence of the vas deferens: incomplete penetrance of cystic fibrosis gene mutations. J. Urol. **158**: 1794-1799.

Short, R. (1995). Human reproduction in an evolutionary context. Ann. N.Y. Acad. Sci. **709**: 416-425.

Silber, S. and Rodriguez-Rigau, L. (1981). Quantitative analysis of testicle biopsy: determination of partial obstruction and prediction of sperm count after surgery for obstruction. Fertil. Steril **36**: 480-485.

Silber, S. (1989). The relationship of abnormal semen parameters to male fertility. Opinion. Hum. Reprod **4**: 947-953.

Silber, S., Nagy, Z., Liu, J., et L., (1994). Conventional IVF versus ICSI for patients requiring microsurgical sperm aspiration. Hum. Reprod **9**:1705-1709.

Silber, S., Van Steirteghem, A., Liu, J., et al., (1995a). High fertilization and pregnancy rates after ICSI with spermatozoa obtained from testicle biopsy. Hum Reprod **10**: 148-152.

Silber, S., Nagy, Z., Liu, J., et al. (1995b). The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. Hum. Reprod **10**: 2031-2043.

Silber, S., Van Steirteghem, A., Devroey, P. (1995c). Sertoli cell only revisited. Hum. Reprod **10**: 1031-1032.

Silber, S., Van Steirteghem, A., Nagy, Z., et al., (1996). Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. Fertil Steril **66**: 110-117.

Silber, S., Nagy, Z., Devroey, P., et al., (1997a). Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testis of men with germinal failure. Hum. Reprod **12**: 2422-2428.

Silber, S., Nagy, Z., Devroey, P., et al., (1997b). The effect of female age and ovarian reserve on pregnancy rate in male infertility: treatment of azoospermia with sperm retrieval and intracytoplasmic sperm injection. Hum. Reprod **12**: 2693-2700.

Silber, S., Alagappan, R., Brown, L. and Page D. (1998). Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. Hum. Reprod **13**: 3332-3337.

Silber, S. (1998a). Intracytoplasmic sperm injection (ICSI) today: a personal review. Hum. Reprod **13**: 208-218.

Silber, S. (1998b). Editorial: The cure and proliferation of male infertility. J. Urol. **160**:2072-2073.

Silber, S. (1999). The disappearing male. In: **Towards Reproductive Certainty - Fertility and Genetics Beyond 1999**. Jansen R, Mortimer D. eds, New York/London: The Parthenon Publishing Group, 499-505.

Silber, S. (2000a). Evaluation and treatment of male infertility. Clin. Ob.Gyn. **43**: 854-888.

Silber, S. (2000b). Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Hum. Reprod. **15**:2278-2284.

Silber,S. (2001). The varicocele dilemma. Hum. Reprod 7:70-77.

Silber, S. Page, D., Brown, L. and Oates, R. (2001). Diverse testicular defects in infertile men with Y deletions and their ICSI offspring. Abstract presented at 2001 Annual Meeting of European Society of Human Reproduction and Embryology, Lausanne, Switzerland.

Simoni, M., Gromoll, J., Dworniczak, B., et al. (1997). Screening for deletions of the Y chromosome involving the DAZ (Deleted in Azoospermia) gene in azoospermia and severe oligozoospermia. Fertil Steril **67**: 542-547.

Sokol, R. and Sparkes, R. (1987). Demonstrated paternity in spite of oligospermia. Fertil Steril **47**:356-358.

Staessen, C., Coonen, E., Van Assche, E., et al. (1996). Preimplantation diagnosis for X and Y normality in embryos from three Klinefelter patients. Hum. Reprod **11**: 1650-1653.

Steinberger, E. and Tjioe, D. (1968). A method for quantitative analysis of human seminiferous epithelium. Fertil Steril **19**: 960-970.

Stuppia, L., Calabrese, G., Franchi, P.. et al., (1996). Widening of a Y-chromosome interval-6 deletion transmitted from a father to his infertile son accounts for an oligozoospermia critical region distal to the RBM1 and DAZ genes. Am. J. Hum. Genet. **59**: 1393-1395.

Stuppia, L., Gatta, V., Calabrese, G., et al. (1998). A quarter of men with idiopathic oligo-azoospermia display chromosomal abnormalities and microdeletions of different types in interval 6 of Yq11. Hum. Genetics **102**: 566-570.

Sun, C., Skaletsky, H., Birren, B., et al. (1999). An azoospermic man with a de novo point mutation in the Y- chromosomal gene USP9Y. Nature Genetics **23**: 429-432.

Sun, C., Skaletsky, H., Rozen, S., et al. (2000). Deletion of azoospermia factor a (AZFa) region of human Y chromosome caused by recombination between HERV 15 proviruses. Hum. Mol. Genet. 9: 2291-2296.

Tiepolo, L. and Zuffardi, O. (1976). Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet, 34, 119-124.

Tilford, C., Kuroda-Kawaguchi, T., Skaletsky, H., et al. (2001). A physical map of the human Y chromosome. Nature **409**; 943-945.

Tournaye, H., Devroey, P., Liu, J., et al., (1994). Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. Fertil Steril **61**: 1045-1051.

Tournaye, H., Staessen, C., Liebaers, I., et al. (1996). Testicular sperm recovery in nine 47,XXY Klinefelter patients. Hum. Reprod **11**: 1644-1649.

Van Assche, E., Bonduelle, M., Tournaye, H., et al. (1996). Cytogenetics of infertile men. Hum Reprod **11**: 1-26.

van der Ven, K., Montag, M., Peschka, B., et. al. (1997). Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. Mol. Hum. Reprod **3**: 699-704.

Van Golde, R., Wetzels, A., de Graaf, R., et al. (2001). Decreased fertilization rate and embryo quality after ICSI in oligozoospermic men with microdeletions in the azoospermia factor c region of the Y chromosome. Hum. Reprod **16**: 289-292.

Van Landuyt, L., Lissens, W., Stouffs, et al. (2000). Validation of a simple Yq deletion screening programme in an ICSI candidate population. Mol. Hum. Reprod 6,:291-297.

Van Steirteghem, A., Nagy, Z., Joris, H., et al. (1993). High fertilization and implantation rates after intracytoplasmic sperm injection. Hum. Reprod **8**: 1061-1066.

Vereb, M., Agulnik, A., Houston, J. et al., (1997). Absence of DAZ gene mutations in cases of non-obstructed azoospermia. Mol. Hum. Reprod **3**: 55-59.

Vermeulen, A., Vandeweghe, M. and Deslypere, J. (1986). Prognosis of subfertility in men with corrected or uncorrected varicocele. J. Androl. 7:147-155.

Vidal, V., Chaboissier, M-C., de Rooij, D.G. and Schedl, A. (2001). Sox9 induces testis development in XX transgenic mice. Nature Genetics **28**: 216-217.

Vogel, T., Speed, R. Teague, P. and Cooke, HJ. (1999). Mice with Y chromosome deletion and educed RBM genes on a heterozygous DAZL1 null background mimic a human azoospermic factor phenotype. Hum. Reprod **14**: 3023-3029.

Vogt, P., Edelmann, A., Kirsch, S., et al. (1996). Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum. Mol. Genet. 5:933-943.

Vogt, P., Affara, N., Davey, P., et al. (1997). Report of the Third International Workshop on Y Chromosome Mapping 1997. Cytogen. Cell Genetic, 79: 1-20.

Vogt, P. (1998). Human chromosome deletions in Yq11, AZF candidate genes and male infertility: history and update. Mol. Hum. Reprod 4:739-744.

Vollrath, D., Foote, S., Hilton, A., et al. (1992). The human Y chromosome: a 43-interval map based on naturally occurring deletions. Science **258**:52-59.

Wang, P., McCarrey, J., Yang, F. and Page, D.C. (2001). An abundance of X-linked genes expressed in spermatogonia. Nature Genetics **27**: 422-426.

Winge, O. (1927) .The location of eighteen genes in Lebistes reticulatus. J Genet, 18, 1-43.

Yen P. (2001) .The fragility of fertility. Nature Genetics **29**: 243-244.

Zukerman, Z., Rodriguez-Rigau, L., Weiss, D. (1978). Quantitative analysis of the seminiferous epithelium in human testicle biopsies and the relation of spermatogenesis to spermatogenesis to sperm density. Fertil Steril **30**: 448-455.