The Disappearing Male

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INTRODUCTION

There is substantial epidemiological debate about an apparent decrease in the sperm count over the past 50 years[1–16]. It has been suggested that such a decreasing sperm count might be due to increasing environmental pollution, estrogens in our diet, differences between geographical regions, or even the anxiety and stress of modern living[17–32]. However contestable this debate about a recent decline in sperm count might be, there is little doubt that, over many millennia, there has been constant genetic and evolutionary pressure towards an inexorable decline in human spermatogenesis.

The human male has just about the worst spermatogenesis of any species on the planet. Most animals produce about 25 million sperm per day per gram of testicular tissue, whereas the human produces only 4–6 million sperm per day per gram of testicular tissue. We are satisfied that a man is fertile when he has greater than 20% normal sperm morphology, but for most animals it would be 'inconceivable' to find more than a few abnormal sperm in the entire ejaculate[33]. The wretched condition of human spermatogenesis has been explained by the concept of sperm competition, with monogamous mating patterns leading to poor spermatogenesis, and promiscuous mating patterns leading to superior spermatogenesis[34]. However, it is only recently, with the mapping of the Y chromosome, that we are developing a better molecular understanding of the poor spermatogenesis of humans, and of the evolutionary pressure down through the ages towards worse and worse spermatogenesis in the human male.

TRANSPOSITION AND COLLECTION OF SPERMATOGENESIS GENES TO THE Y CHROMOSOME

After the first successful mapping of a complete chromosome, i.e. the Y chromosome, with sequence tagged sites (STS) markers, it became possible to study a hypothesis that began in the 1920s about the translocation of sperm production genes from various regions of the genome to the Y chromosome[35,36]. In virtually all species, significant defects in the Y chromosome lead to a complete failure of spermatogenesis with no other obvious genetic abnormalities. This phenomenon has been referred to as 'pure sterile' genes, presumably located on the Y chromosome of most, if not all, species. There are undoubtedly many spermatogenesis genes located throughout the genome, but over 250 million years there has been a continual process of evolution of the Y chromosome with the deterioration of existing spermatogenesis genes located *on* the Y and transposition of new spermatogenesis genes *to* the Y via a process referred to as the collection of 'sexually antagonistic genes'[37–46].

It was originally postulated in the early 1920s (before the Y chromosome was understood – in experimental work with multicolored guppies) that genes that are beneficial to the male sex and disadvantageous to the female sex tend to accumulate near a testicular determining factor (*TDF*) gene[45]. This area becomes a 'safe harbor' for 'sexually antagonistic genes' favorable to the male and unfavorable to the female[47,48]. Now, with modern Y chromosomal mapping techniques, we have been able to determine that the Y chromosome contains at least 12 testis-specific genes that are transcribed only in the testes, and

presumably play an important role in spermatogenesis, thus verifying this 1920s' theory[37].

THE Y CHROMOSOME IN INFERTILE PATIENTS

When we first began to perform Y chromosomal mapping on azoospermic and severely oligospermic males in 1993, we found that in 13% of men with non-obstructive azoospermia there were clearly identifiable microdeletions in the Y chromosome that were not present in their fathers, their brothers, or over 100 fertile male controls[43]. Seven per cent of severely oligospermic men had similar microdeletions, but no patients with greater than 5 million sperm/ml had any microdeletions detected[49]. Furthermore, below this limit the severity of the spermatogenic defect, i.e. whether or not we were able to retrieve sufficient sperm to perform intracytoplasmic sperm injection (ICSI) either from the ejaculate or from the testis (testicular sperm extraction, TESE), was related to the size of those Y chromosome microdeletions, and whether or not they encompassed more than one specific gene locus on the Y.

We still did not have a molecular diagnosis for the cause of the spermatogenic defect in the other 87% of men with non-obstructive azoospermia, perhaps because molecular methods for finding small mutations on the Y chromosome were hampered by the presence of multiple repeat copies of the same genes, so-called gene clusters. Therefore, many other mutations in testis-specific genes of the Y chromosome are likely to be evading our detection. Nonetheless, we now have the evidence that spermatogenesis, to a significant degree in the human male, is controlled by a variety of genes in multiple copies on the Y chromosome[37,49,50].

The first gene identified, and the gene most commonly deleted in our azoospermic and severely oligospermic men, we termed *DAZ* for 'deleted in azoospermia'[43,44,50]. This *DAZ* gene represented the smallest of all the microdeletions found in what has now been termed the AZFc region of the Y chromosome[39]. When deletions extended beyond this small AZFc region, no spermatogenesis whatsoever was detected[49]. When the deletions were limited to this AZFc region, there were tiny amounts of spermatogenesis that would have previously led to sterility, but, with testicular sperm extraction and ICSI allowed these men to father children genetically their own[39,49,51–56]. The *DAZ* gene is the gene our group has studied most extensively, and which gives us the beginning of an understanding of how the Y chromosome evolved and how this evolution could be an explanation for the poor quality of spermatogenesis in the human male.

EVOLUTION OF THE Y CHROMOSOME

After *DAZ* was identified as an important spermatogenesis gene deleted in the AZFc region in the majority of our patients with Y deletions, homologs for this Y chromosomal *DAZ* gene in the human were sought in the rest of the human genome, as well as in lower animals[41,43,44]. An autosomal homolog of the human *DAZ* gene was found on human chromosome[41,57]; on chromosome 3 there is only one copy, whereas on the Y, there are multiple copies of the gene. The *Drosophila* spermatogenesis gene, named *Boule*, a homolog of the human *DAZ* gene that we found deleted in our azoospermic men, was found to be required for spermatogenesis in the fly[42]. Loss of *Boule* function in flies blocks meiotic divisions and causes azoospermia, similar to what is seen in humans with AZFc deletions. Boule, like *DAZ*, was found to be a 'pure sterile' gene, meaning that fruit flies that lack this gene were normal in every other way except that they were infertile. This gene in the fruit fly was found to be autosomal, a single copy, and not located on the Y chromosome. In a similar fashion, a mouse autosomal homolog of the *DAZ* gene, called *DAZLA*, is located in a single copy on chromosome 17 (again not on the Y chromosome)[40,58]. On the other hand in apes, homologs of the human *DAZ* gene are located on the Y chromosome, where, similar to the human, they exist in multiple copies.

There is no evidence of a *DAZ* homolog on the Y chromosome of mice, rabbits, dogs or cattle[59]. These animals carry a single autosomal homolog of *DAZ*. Despite the usual

deterioration of genes on the Y, *DAZ* genes have maintained their exon–intron structure. (Other gene families located on the Y chromosome no longer contain introns and exons, including other genes in the AZFc region.) Study of the *DAZ* genes in various animals including humans will serve as the best model we currently have for understanding the evolution of the Y chromosome and spermatogenesis.

DAZ genes were evidently transposed to the Y chromosome during primate evolution[41,59]. Below the primates, *DAZ* genes are not found on the Y. The ancestral *DAZ* gene, often referred to as *DAZL* (*DAZ*-like), is found autosomally on chromosome 3 in humans, and has autosomal homologs in all other organisms studied including flies, frogs, worms and most mammals[37,41,59]. Disruption of these autosomal *DAZ* genes in flies and mice causes dosage-dependent infertility. That is, mice who are homozygously knocked out for *DAZL* are completely sterile, but heterozygous mice with one copy of the *DAZL* gene intact simply exhibit reduced spermatogenesis, not an absence of spermatogenesis[40]. Thus, the number of copies of the *DAZ* or *DAZL* gene, at least based on animal studies, could quantitatively affect the amount of spermatogenesis. Furthermore, we have found that men who lack only the *DAZ* gene cluster, but in whom the rest of the Y chromosome is intact, have either severe oligospermia or azoospermia with some sperm retrievable from the testes. Absolute azoospermia even after meticulous testicular exploration is only found when the Y deletion extends beyond the *DAZ* region[39,49,60,61].

When the *DAZ* gene is found in the safe environment of autosomal chromosomes that undergo recombination, it exists in a single copy. Once it is transposed to the Y, which occurred during primate evolution, these *DAZ* genes amplify in number and prune their exons within the transposed genes and develop multiple copies[41]. Other genes on the Y in humans clearly related to spermatogenesis, such as *RBM*, are also found on the Y chromosome in multiple copies but in different regions of the Y[39,54,56,59,60,62]. The acquisition of formerly autosomal fertility genes by the Y chromosome has been an important process in Y chromosome evolution.

X and Y chromosomes arose independently in many evolutionary lineages, in each case deriving from an ordinary autosomal pair[47,48,59]. Once the recombination between the developing X and Y chromosomes becomes limited, a necessity for the development of a testis-specific gene, the gene content of the Y chromosome declines steadily and inexorably[38]. Degeneration of the Y chromosome is well documented in fruit flies, and is an ongoing process in all animals. The few genes that remain on the Y chromosome are either relics of a common ancestry with the X chromosome (and have ubiquitous cellular functions) or are relatively new testis-specific genes that have been accumulated from the rest of the genome: such 'incipient Y chromosomes' accumulate alleles that enhance male fitness but diminish female fitness. Sexually antagonistic or 'male benefit' genes are thus acquired on the Y chromosome, which tends to accumulate genes, therefore, that enhance spermatogenesis[37].

Although the *DAZ* gene arrived at the Y chromosome via a transposition from autosomal *DAZ*, and then underwent amplification on the Y, the *RBM* spermatogenesis gene has a slightly different history. It began on the ancestral X–Y chromosome pair (when they were still autosomal), and then amplified and degenerated on the Y as the ancestral Y chromosome evolved. Either way, the implications are the same. The study of the *DAZ* gene family in various animals and in humans helps us to understand better the evolution of the human Y chromosome, and ultimately why humans have such terrible spermatogenesis, compared to other animals. Presumably, all spermatogenesis genes over time undergo a similar evolutionary history as *RBM* and *DAZ*.

THE Y CHROMOSOME IS *NOT* A SAFE PLACE

The Y chromosome, although a safe harbor for transposition of autosomal spermatogenesis genes over many millions of years, is nonetheless, in the long run, a dangerous place

for any genes because of the lack of recombination[47,48,59]. The evolution of the X and Y chromosomes, from what were originally two autosomal paired chromosomes over 250 million years ago, gives us one clue to the inexorable decline of the human spermatogenesis. One starts 250 million years ago with two ordinary autosomes, and one of those autosomal chromosomes, for whatever reason, develops a *TDF* gene, with a failure of recombination and crossover[46]. Because of the failure of recombination of that pair of chromosomes, which is needed for the *TDF* gene to efficiently retain genomic integrity, the chromosome that does not cross over in either male or female meiosis inevitably undergoes informational degeneration. Failure of recombination does not give a chromosome a chance to repair itself, and it degenerates and shrinks. The complementary chromosome to this new Y chromosome (i.e. the new X chromosome) is protected from such shrinkage and deterioration because in the female germ cells it pairs fully at meiosis and does have a chance to repair itself.

Although most of the human genome represents a scattered mosaic of genes randomly distributed among the various chromosomes, the Y chromosome demonstrates a remarkable functional coherence[37]. The Y chromosome consists of two components. One component is the remnant of the thousand or so X homologous genes that escape X inactivation. These are the so-called Turner's genes. There appear to be probably eight definable such genes in the non-recombining portion of the Y chromosome (NRY) that escape X inactivation and have general cellular housekeeping functions not specific to the testis. On the other hand, there are 12 testis-specific genes on the non-recombining portion of the Y that are not necessary at all for the overall proper functioning of the organism, but which are necessary for proper testicular function. Thus, in the process of the deterioration of the Y chromosome with the loss of most of its X homologous genes there is an accumulation of genes specifically necessary for testicular function[37]. There is a dynamic evolutionary process whereby spermatogenesis genes on the Y chromosome are lost owing to degeneration of the Y, and replaced by new spermatogenesis genes transposed from autosomal locations[59]. Once a spermatogenesis gene is transposed to the safe harbor of the Y, it undergoes multiple amplifications and pruning. Because of the inevitable deterioration of the Y chromosome, where mutations and deletions occur at a much faster rate than in autosomes because of failure of recombination, this is a treacherous location for genes that are so important for the continuation of the species. This downward pressure on spermatogenesis is true for *all* animals.

MALE-TO-MALE COMPETITION

What protects most species from gradual extinction of the male (and therefore of the species) is the phenomenon of sperm competition. This concept, popularized by Roger Short, is that, throughout the animal kingdom one can predict the size of the testicle, and possibly thereby the quality of spermatogenesis, by the mating pattern of the species[33,34]. Species that have monogamous mating patterns or bigynous mating patterns (where there is either one male for one female or one male for several females) have no sperm competition. If the male of the family unit has a low sperm count and the female has no social choice but to copulate with this male, then should she become pregnant the offspring will inherit the low sperm count of the father. This is, for example, the mating pattern in gorillas, who have tiny testicles and extremely low sperm counts apparently with poor sperm quality. However, chimpanzees have relatively enormous testicles and are extraordinarily fertile, despite having similar Y chromosomal spermatogenesis genes to gorillas. Chimpanzees have a 'promiscuous' (in reality, polyandrous) mating pattern, in which a female in a troop of 30–50, (living together as a large family unit) in heat might be mounted by every single male. Thus, with all the sperm that she encounters as a result of her many copulations during heat the one that is most likely to result in a pregnancy will on average be the sperm that comes from the most fertile male. That would mean that a male offspring of the female chimpanzee is more likely to have very good

spermatogenesis than a male offspring of a female gorilla. Humans are closer to the gorilla than the chimpanzee, in terms of both spermatogenesis and mating pattern.

This association can be found throughout the animal kingdom. Geese, who mate for life, have small cryptorchid testicles, but turkeys, which have a polyandrous mating pattern, have very large cryptorchid testicles. In other species such as the antlered deer and sheep, the males undergo physical battle to determine which one will mate with the female[63]. Large testicles and a high testosterone level give an indirect advantage to the male with the greatest sperm production. Thus, throughout the animal kingdom, the evolutionary pressure towards a decrease in sperm count with successive generations, because of the precarious nature of the Y chromosome is offset by the phenomenon of sperm competition, which weeds out of the population the Y chromosomes that have already undergone degeneration resulting in decreased spermatogenesis.

GENETIC TRANSMISSION OF INFERTILITY TO MALE OFFSPRING

A missing link in this theory of the declining sperm count and the evolution of the Y chromosome, has been the issue of whether these mutations in the Y chromosome and these deletions of spermatogenesis genes found in sterile males are transmitted to succeeding generations via ICSI[64]. For example, just because an infertile male has a Y chromosomal deletion resulting in decreased spermatogenesis does not necessarily mean that the few sperm he *is* making have a Y deletion. It would have been conceivable to think that the areas within the deficient testicle where normal spermatogenesis is actually occurring in these infertile men with very low sperm production could be mosaic regions where there is a normal Y chromosome. In 1995, after demonstrating these Y deletions in azoospermic men, we suggested that such deletions could be transmitted to the male offspring of couples undergoing *in vitro* fertilization (IVF) and ICSI for severe male factor infertility[65]. This was speculation at the time, but we now have data that confirm that in azoospermic and severely oligospermic men undergoing ICSI the exact Y deletion of the infertile father is indeed transmitted to his male offspring[49,66].

It is important to remember that there are autosomal spermatogenesis genes throughout the genome that have not yet been identified. It should also be noted that there are some cases of severe oligospermia and azoospermia that are related to grossly demonstrable chromosomal aberrations (1.3%) such as translocations and sex chromosomal aneuplodies such as Klinefelter's syndrome (0.2%). Inbreeding studies in exotic species by O'Brien and others[67,68] have highlighted the severely negative effect on spermatogenesis of having a highly inbred population with low genetic diversity. This could be explained by the failure of sperm competition by the accumulation of autosomal recessive spermatogenesis defects.

THE DISAPPEARING MALE

The course of many millions of years of evolution, and the precarious nature of the environment of the Y chromosome because of the absence of meiotic recombination, has made spermatogenesis an evolutionary fragile phenomenon. Its reversal, and thus the prevention of the extinction of the male of a species, depends on a mating system that ensures sperm competition.

As humankind benefits from a strong family system and a monogamous mating pattern, we have a conflict[63]. Progress of humanity, with regard to science, culture and morals, appears to depend on a strong, loyal, monogamous family mating system, which fosters the transmission of knowledge and ideas from one generation to the next. We would not be sending men into space, and solving the riddles of disease, and providing the technology for food production to feed everyone on the planet, were it not for the intellectual and cultural evolution of our species. This is fostered by the educational benefits of a strong family system promoted by monogamous mating. Human babies are born without the ability to survive or develop through inborn, instinctive abilities. We have a relatively gigantic brain capacity and a rather feeble body, so as children we must be nurtured and

educated by our parents for many years before we become independent. Not only would the human race not develop culturally without the education afforded by a strong family system, but indeed, as we see in many unfortunate socio-economic circles deprivation produces much human wastage. The irony is thus that the family system and the monogamous mating pattern our culture as humans depends on look like guaranteeing of our eventual reproductive disappearance.

The force for extinction of the male is evident even in the cloning experiments of Yanagimachi on rodents, which do not work for Sertoli cells but do work for cumulus cell injection[69,70]. It thus appears, at least in his model, that females can be cloned, but males cannot. Not that cloning is any solution to the cosmic dilemma written about in this chapter; but it is a humorous side-note that, everywhere we turn in reproductive science the continued existence of the male is under threat!.

Ironically also, the development of technology that now allows severely oligospermic and azoospermic men to father children is likely to increase the prevalence in our population of infertile males, and thus accentuate the problem of the declining sperm count in a way that may become more measurable in the next few generations[49,66,71]. Can we afford to continue our monogamous ways, and can we afford to continue to allow infertile men to have children who will also be infertile? As there has been no major increase in genetic or congenital abnormalities in the offspring of these infertile men (other than the likelihood of their being infertile), prospective parents in my experience are universally unconcerned about this issue. If their son has the same problem they have and they are able to have offspring, they figure that, in 25 years, it will be even easier for their son to have a child than it was for them! Furthermore, our studies do demonstrate that the Y deletion of the infertile parent is not amplified in the offspring, but is transmitted identically. Thus, there is no reason to believe that the infertile male offspring will not be able to have a child just as readily as his father did.

Nonetheless, we would expect that in 20 years ICSI will be considered a barbaric and crude procedure of the 20th century. In the 21st century, we could anticipate a direct molecular approach to treating male infertility that will ultimately allow couples once again to have children conceived in their bedroom, rather than the laboratory[64]. In summary, Y chromosome research has led to a better clinical understanding of male infertility, and a better understanding of the entire evolution of the Y chromosome, but has also revealed an inexorable evolutionary pressure towards extinction of the male.

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