



## Microsurgical solutions to male infertility

By Sherman J. Silber, MD

What causes male infertility? Look to the Y chromosome, says this leading expert. Among the high-tech solutions worth considering: testicular sperm extraction (TESE), microsurgical epididymal sperm retrieval (MESA), and intracytoplasmic sperm injection (ICSI).

**B**ecause most defects in sperm are actually genetic in origin, conventional therapy for male infertility would seem doomed to fail.<sup>1</sup> Only 10 years ago, sterile men had no hope of ever fathering a child—until modern microsurgical techniques revolutionized the field of infertility. We discussed interpreting semen analysis in the October 2004 issue of this magazine. In Part 2 of this two-part series, I'll discuss genetic causes of infertility, specifically chromosomal errors revealed by the recent sequencing of the "Y" chromosome. We'll also explore the latest techniques to circumvent these defects, including testicular sperm extraction (TESE), microsurgical epididymal sperm retrieval (MESA), and intracytoplasmic sperm injection (ICSI). The latter—performed after one of these types of sperm retrieval—is now the most effective treatment for even the most severe cases of male infertility.<sup>2</sup>

### **Karyotype abnormalities**

Chromosomal errors abound in sterile men, as an analysis comparing karyotypes of infertile men

with normal newborns has shown.<sup>3</sup> In fact, the incidence of *balanced translocations* (see glossary) in infertile males (about 2%) is more than four times that found in normal newborns (0.25%).<sup>3</sup> Some type of autosomal chromosome anomaly—either balanced Robertsonian translocations, balanced reciprocal translocations, balanced inversions, or extra markers—is found in almost 3% of oligospermic males (those having low concentrations of sperm in the ejaculate). Sex chromosomal anomalies, such as Klinefelter's, are found in about 4% of azoospermic men (see glossary).

Since 2% of oligospermic infertile males have chromosomal translocations (compared to 0.25% in a control population), it should come as no surprise that 0.9% of ICSI offspring inherit such a translocation from their fathers.<sup>4,7</sup> But it's somewhat reassuring that only 10% of those inherited translocations were unbalanced (only 0.1% of ICSI offspring). However, the other translocations that were transmitted in a balanced fashion, with "normal" offspring, are very likely to have the same

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DR. SILBER, a pioneer in microsurgery and infertility, is a leading authority on IVF, egg and embryo freezing, sperm retrieval, and ICSI. He performed the world's first microsurgical vasectomy reversal, the first testicle transplant, and first ovary transplant. The author of four best-selling books for the layman as well as over 250 scientific papers on infertility and reproduction, he is Director of the Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, Mo.

## Take-home messages

- As long as their spouses don't have serious fertility problems, most men with azoospermia can have healthy children using the sperm retrieval method of testicular sperm extraction (TESE) along with intracytoplasmic sperm injection (ICSI).
- All of the ICSI-derived sons of these infertile men have been shown to carry the same Y chromosome deletions as their infertile fathers.
- Provide genetic counseling to all infertile males, even if no abnormality is detected or a Y deletion assay isn't done. Routine lab studies pick up only a fraction of the genetic causes of male infertility.
- The author's microsurgical TESE technique avoids the potential damage of multiple biopsies and a "hit or miss" approach. It minimizes tissue loss, damage, pain, and permits future attempts at retrieval.

infertility defect as the offspring's fathers (0.83%). Based on crude cytogenetic studies, we can therefore anticipate that almost 2% of ICSI offspring will be infertile or sterile, which is over fivefold what you'd expect in normal newborns.

Even so, the karyotypic study of these offspring is more reassuring than alarming. Fortunately, the incidence of congenital abnormality in ICSI children is no greater than in every normal population studied.<sup>4,6</sup>

Even the few reported ICSI offspring of Klinefelter's patients have been chromosomally normal. Arguably, there's no greater incidence of autosomal aneuploidy following ICSI than one would predict from maternal age. Even sex chromosome aneuploidy (0.7%) in ICSI offspring, which is four times that of controls, isn't really very worrisome. Thus, despite the possible occurrence of infertility and sex chromosomal disorders in a very small percentage of cases, the evidence from cytogenetic and pediatric follow-up of ICSI offspring is otherwise quite reassuring. Molecular study of the Y, however, is of far greater concern regarding the future fertility of these children.

### Y chromosome deletions

Using molecular chromosomal mapping techniques (which have much greater resolution than cytogenetics), we originally reported microdeletions encompassing the AZFc region of the Y chromosome (a critical sperm production region) in 13% of azoospermic men and 7% of severely oligospermic men.<sup>8</sup>

However, these Y deletions may represent only the tip of the iceberg, since even these subtle "microdeletions" on the Y chromosome represent gross dropouts of many hundreds of thousands of nucleotides. (GR-GR deletion takes out only half of the genes in the AZFc region, and therefore results in lesser degrees of oligospermia. Thus, most GR-GR deletions are transmitted naturally, whereas it is very rare for the larger AZFc deletion to be transmitted naturally.) The usual molecular mapping methods cannot yet pick up smaller mutations. That's why we made such a huge investment in actually sequencing every nucleotide of the Y chromosome, not just settling for crude mapping, as many labs currently perform on the Y chromosome.<sup>9,10</sup>

Clearly, ICSI's current success in treating male infertility will lead to greater infertility in future generations.<sup>11-15</sup> However, the X chromosome and the autosomes are also involved. There are at least nine testis-specific gene families and 60 genes located on the Y chromosome that interact to affect spermatogenesis; and this most likely represents only one third of male germ cell-specific genes.

Many are on chromosomes other than the Y. Another one third of testis-specific germ cell genes are likely on the X chromosome.<sup>16</sup> Because of the multiple copies that exist for most of the genes located on the Y, smaller point mutations, which may be much more common than these reported "microdeletions," are naturally much more difficult to find. Nor would current

sequence tagged site (STS) mapping pick up smaller (or partial) deletions of major regions of Y, yet they've been found in abundance by actual sequencing of the Y chromosome.<sup>10,11,17,18</sup>

#### **Why did sperm-producing genes move to the "Y" chromosome?**

Over the course of the last 300 million years of mammalian evolution, the X and the Y chromosomes have evolved from what were originally two ordinary autosomes. During that evolution, spermatogenesis genes have transposed (or retro-positioned) themselves from autosomes to Y, and there amplified into multiple palindromic copies.<sup>19-21</sup> These spermatogenesis genes include *DAZ* and *CDY*, but we've discovered many more (at least 60) by actually sequencing Y. Some spermatogenesis genes—*RBM*, for example—have persisted from their original position on X and achieved greater prominence on Y. Indeed, even the *SRY* gene (the male sex-determining locus) began on the ancestral X chromosome. *Genes associated with the nonrecombining SRY region on the evolving Y chromosome that were specifically beneficial for male function flourished there as a "safe harbor."*<sup>22-29</sup>

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" genes that otherwise would be hidden throughout the genome. Since most of the Y chromosome is not subject to meiotic crossover events such as occur in

## Glossary

**Azoospermia:** A cause of male infertility in which no sperm are present in the ejaculate.

### Balanced vs. unbalanced translocations:

**Unbalanced** translocation: there is either too much or too little chromosomal material; it's caused by a part of a chromosome being broken off from its usual location. Such translocations are either lethal or cause abnormalities.

**Balanced** translocation: there is a piece of one chromosome located on another chromosome, but there is no obvious genetic material missing. Such "balanced" translocations are found in normal individuals, but their meiosis is abnormal and offspring may suffer "unbalanced" translocations.

**Obstructive azoospermia:** The type caused by vasectomy, congenital absence of vas, epididymal scarring from infections.

**Nonobstructive azoospermia:** The absence of sperm in the ejaculate, which results from low or seemingly no sperm production, so that no sperm reach the ejaculate.

**ICSI (intracytoplasmic sperm injection):** A microsurgical IVF procedure that follows sperm retrieval (usually via TESE or MESA), in which a single sperm cell is injected directly into the cytoplasm of an egg to help overcome male factor infertility.

**MESA (Microsurgical Epididymal Sperm Aspiration):** A microsurgical technique for obtaining sperm from the epididymis that enables individual epididymal tubules to be dissected under direct vision and the proximal most sperm with the best motility to be retrieved in cases of obstructive azoospermia.

**Oligospermia:** Deficiency in the number of sperm in the ejaculate, causing male infertility.

**Palindrome:** A sequence of DNA or RNA that reads the same in either direction. New research shows that large sections of the Y chromosome repeat in reverse order.

**TESE (testicular sperm extraction):** TESE is a microsurgical approach that avoids the need for multiple biopsies from every area of the testis and the risks of invasive procedures. It consists of opening the vaginalis, and exteriorizing the testicle before using the operating microscope. After microdissection and analysis via tubular dilation, numerous sperm can often be microscopically retrieved through just a single dilated tubule.

all other chromosomes, molecular defects cannot be readily repaired. This "instability" of the Y chromosome suggests an inexorable decline in sperm production in the evolution of any species with a Y-chromosome equivalent, unless there's either sperm competition within the species' mating pattern or new spermatogenesis genes are continually recruited to the Y chromosome with subsequent amplification before ultimately degenerating.<sup>19,20,26</sup>

Because of the effectiveness and widespread adoption of ICSI, ster-

ile men are now becoming fathers. All of the ICSI-derived sons of these infertile men have been shown to carry the same Y chromosome deletions as their infertile fathers.<sup>11,12,14,26,30</sup>

Clearly, a negative Y microdeletion assay doesn't rule out genetic abnormality. Furthermore, most causes of nonobstructive azoospermia are likely related to genetic abnormalities that current routine lab testing isn't sophisticated enough to pick up. Therefore, in my view, genetic counseling should be provided to all infertile males,

whether an abnormality is detected and whether labs even bother to do Y deletion assays. Currently, routine lab studies pick up only a fraction of the causes of male infertility, most of which clearly must be genetic.

Interestingly, our scrutiny of the "Y" chromosome seems to have also shed light on a scientific mystery of another kind (see "Human male infertility and dinosaur extinction").

### The accumulation of testis-specific genes on "Y"

There's a parallel accumulation of genes on the Y chromosome that control sperm production, along with the decay of most of the ancestral autosomal genes on the heterogametic sex chromosome controlling GSD (the Y chromosome in mammals). That's inevitable because the region adjacent to the testis-determining gene—one that doesn't recombine during meiosis—provides a "safe harbor" for genes that are helpful to the male but detrimental to the female. These "sexually antagonistic" male-benefit genes enhance male fertility because they're testis-specific. And they've been accumulating and amplifying on the nonrecombining region of the Y for 300 million years.

In this way, a functionally coherent concentration of testis-specific genes has arisen on a labile Y chromosome that's subject to deletions and inversions caused by massive direct and inverted regions of nucleotide identity (amplicons and palindromes), and is a significant cause of human male infertility. A

## Human male infertility and dinosaur extinction

Serendipitously, while studying the sex chromosomes to better understand the molecular genetics of male infertility, we stumbled upon the probable mechanism of dinosaur extinction.<sup>1,2</sup> While a full discussion of the topic is beyond the scope of this article, essentially we looked at the evolution of temperature-dependent sex determination (TSD) and genetic sex determination (GSD). Evolution favors the eventual and repeated re-appearance of GSD because animals (like dinosaurs) who don't use this mechanism are at risk of extinction due to a skewed sex ratio that massive global environmental temperature changes could bring about. We found that the drawback to an evolving sex-determining chromosome (which guarantees a balanced sex ratio) is its eventual decay due to the lack of meiotic recombination during gametogenesis—and in humans, the subsequent loss of genes that trigger sperm production, causing male infertility.<sup>3-5</sup> We've spelled this out in male infertility studies and with the complete sequencing of the Y chromosome.<sup>6-10</sup>

Despite the protection that GSD affords from global temperature shifts, there's a rise in male infertility because of the accumulation on the Y chromosome of a dense concentration of spermatogenesis genes in a chromosomally unstable region (because of failure of chromosomal recombination during meiosis).

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delicate balance exists between "gene conversion" with chromosome repair by recombination between the palindrome arms and their frequent deletion due to a mistaken homologous recombination between massive ampliconic repeat sequences on Y. That's why human males (whose mating

behavior involves scant sperm competition to weed out deleted chromosomes) have such poor sperm production.

The recurring, independent emergence of sex determination based on genes located on sex chromosomes is a foolproof way to ensure a balanced ratio of males to

females in subsequent generations—despite the risk to fertility. The potential reduction in sperm production due to deletions of the shrinking Y chromosome (in the absence of sperm competition) is balanced by the assurance of a stable sex-ratio, protected from shifts in environment.<sup>21,31-39</sup>

### Sperm retrieval and ICSI

Congenital absence of the vas deferens (CBVAD) afflicts about 1% of infertile couples.<sup>40</sup> Up until the last decade, it was a frustrating and dismal problem with very poor prognosis for treatment. Ever since the first successful use of MESA and IVF for CBVAD, ICSI has now made it possible for all of these men to father children.<sup>40-42</sup> In fact, with ICSI, the pregnancy rate with MESA is related only to female factors.<sup>41,43,44</sup>

There's virtually no case of obstructive azoospermia that cannot be successfully treated with sperm retrieval methods and ICSI, as long as the woman herself doesn't have insurmountable fertility problems. For obstructive azoospermia we prefer to use epididymal sperm, although testicular sperm works just as well. The advantage of epididymal sperm is that it freezes so easily and represents such a simple, clean, easy, and indefinite supply of sperm for the laboratory to use for that particular patient, without any need for future invasive procedures. To sum up, it's better than testicular sperm for three reasons: (1) You get more sperm with better motility that therefore freeze better, for liter-

ally an unlimited supply for any future ICSI procedure; (2) It's much easier for the lab, requiring essentially *no* prep at all; (3) In truth, it's less painful than "invading" the testes.

### **MESA**

Microsurgical epididymal sperm aspiration is performed entirely under local anesthesia. In fact, the patient can watch the entire procedure on a video monitor. Both the cord and the anterior scrotal skin are infiltrated with marcaine using a 27-gauge needle. A small anterior scrotal window incision is carried down to the tunica vaginalis, and the epididymus is exposed under the operating microscope. It is important to retrieve sperm most proximally, either from the caput or even from vas efferentia, because in an obstructed state the distal sperm will be most senescent and the proximal sperm the most recently produced with the best motility. Poorly motile distal, senescent epididymal sperm do not give results as good as a more proximal sperm.

### **Microsurgical TESE**

Experts continue to debate the best way to collect epididymal or testicular sperm for ICSI from patients with obstructive azoospermia. There's no doubt, however, about the best approach for nonobstructive azoospermia (NOA). There are a minute number of sperm sparsely present in an extensive testicular biopsy of most such patients, and these occasional testicular sperm

can be used for ICSI.<sup>42,44-47</sup> We first coined this procedure testicular sperm extraction (TESE), basing this approach on quantitative studies of spermatogenesis dating back to the late 1970s.<sup>48-52</sup> Examination of the testicular histology of azoospermic, oligospermic, and normospermic men shows that the number of sperm in the ejaculate is directly correlated to the number of mature spermatids found quantitatively in the testis.

If the testis are producing extremely small amounts of sperm, the ejaculate will contain no sperm at all.<sup>53</sup> A minimum threshold of sperm production is necessary before any sperm can actually spill over into the ejaculate. With this in mind, severe oligospermia, which is readily treated with ICSI, is just a quantitative variant of azoospermia. Actually, there's minute production of sperm in 60% of azoospermic men.

**Hit-or-miss sperm collection is outdated.** The initial approach to TESE for nonobstructive azoospermia was very crude, often involving numerous extensive biopsies from several areas in the testis until sperm were located.

When surgeons perform extensive multiple biopsies from every area of the testis in an effort to find sufficient sperm for TESE, a great deal of testicular damage can result and that may limit "successful" patients to only one attempt.<sup>54,55</sup> And while well-intentioned, efforts to minimize damage by using multiple needle sticks rather than open biopsy to obtain sperm for ICSI are just as invasive and quite risky as

well.<sup>56</sup> Furthermore, controlled studies have shown that for difficult cases of nonobstructive azoospermia, where spermatogenesis is very meager, needle biopsy is much less likely to find the rare foci of spermatogenesis than open biopsy.<sup>57,58</sup>

We studied the distribution of spermatogenesis in azoospermic men and have outlined a *microsurgical* approach to TESE that minimizes tissue loss and pain and makes it very easy to repeat TESE for an indefinite number of cycles. Knowledge of the distribution of spermatogenesis and of microsurgical technique helps to prevent testicular damage and postoperative pain, making multiple repeat TESE procedures safe and reliable.<sup>45,59</sup>

**Using a targeted approach.** All microsurgical TESE cases are performed under local anesthesia. Just like for MESA or for diagnostic testis biopsy, the procedure is truly painless. After opening the tunica vaginalis and exteriorizing the testicle, we then use the operating microscope under 16 to 40 $\times$  magnification. After microdissecting and evaluating how dilated each specific seminiferous tubule is (the more dilated, the more sperm are present), we can often retrieve large numbers of sperm just by removing a single dilated tubule microscopically (Figure 1).

After meticulous hemostasis with microbipolar forceps (Figure 2), the tunica albuginea is closed with 9-0 nylon interrupted sutures. This prevents any increase in intratesticular pressure, resulting in

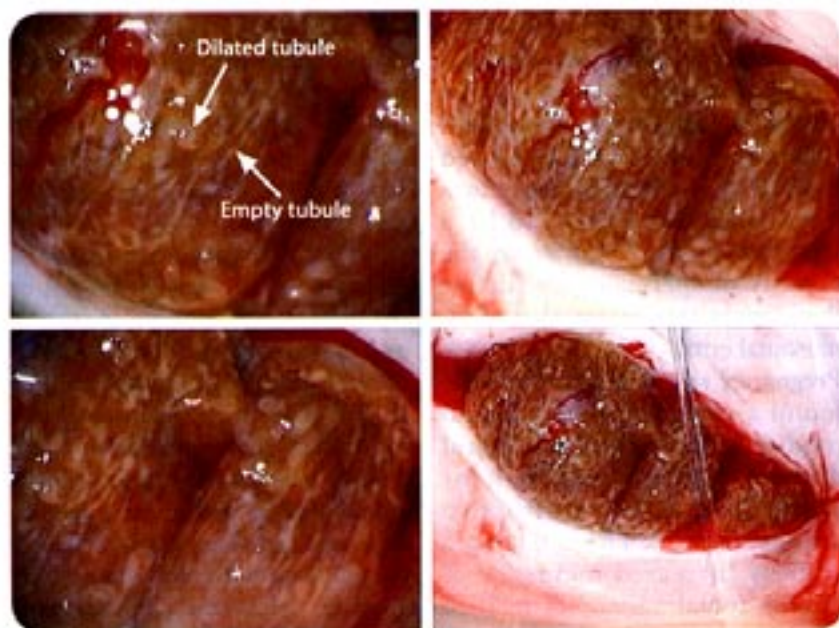
minimal pain, no subsequent atrophy, and no testicular damage.

#### **Chromosomal errors, embryo quality, and more**

FISH analyses of embryos from ICSI cycles for nonobstructive azoospermia (requiring TESE) versus ICSI cycles for oligospermia or obstructive azoospermia show that embryos derived from TESE have a significantly higher rate of chromosome mosaicism.<sup>60</sup> For example, ICSI cycles with ejaculated sperm produced 830 embryos, of which 41.8% were normal, 26.2% were aneuploid, and 26.5% were mosaic. In contrast, ICSI cycles with TESE for nonobstructive azoospermia produced 100 embryos of which only 22% were normal, 17% were aneuploid, and 53% were mosaic. That's a highly significant difference in mosaicism rate between the two groups ( $P < 0.001$ ).

Most of the mosaic embryos were chaotic. (Chromosomally chaotic embryos display a variety of chromosomal abnormalities in different blastomeres [cells] that is different from classic aneuploidy, (like trisomies). Furthermore, these chaotic embryos are more likely to result in embryos with visibly abnormal morphology, such as unequal-sized cells and extensive fragmentation.) Sperm derived from TESE for nonobstructive azoospermia have a higher rate of compromised or immature centrosome structures, and this may be responsible for increased rates of chaotic chromosome errors in the subsequently derived embryos.

In contrast, for oligospermia, the



**FIGURE 1.** Microsurgical testis biopsy performed under 16 to 40 $\times$  magnification shows just one tiny, microscopic, dilated tubule after microdissection and evaluation of tubular dilation.



**FIGURE 2.** A single large testicular incision for a TESE procedure under the operating microscope results in minimal-to-no testicular damage, minimal-to-no postoperative pain, and enables the surgeon to analyze each specific seminiferous tubule for the presence of spermatogenesis.

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rate of chromosome abnormalities found in embryos produced by ICSI is similar to that found with conventional IVF. So the good news is that the ICSI procedure itself does not appear to be a teratogenic agent.<sup>61,62</sup> It's only the severity of spermatogenic defect that causes this increase in chromosomal error.

**Pregnancy rates.** Researchers have found a modestly lower clinical pregnancy rate for testicular sperm derived from men with non-obstructive versus obstructive azoospermia.<sup>63</sup> There is also a slightly increased incidence of chromosomal anomalies in ICSI offspring compared to normal newborns.<sup>64,68</sup> In addition, there are more abnormalities found in peripheral lymphocytes of males who require ICSI.<sup>3,69-74</sup> Even if the infertile male is chromosomally normal in his peripheral lymphocytes, meiotic disruption nonetheless generates higher rates of sperm chromosome abnormalities.<sup>75</sup> Thus, these few sperm in TESE cases have a higher rate of chromosomal errors. Nonetheless, if the wife is fertile and has many eggs, this sperm problem can be overcome and good pregnancy rates still obtained, because there will usually be (out of a large group) some normal embryos.

**Sperm aneuploidy.** We've observed a slightly higher rate of aneuploidy in testicular sperm from men with nonobstructive azoospermia than in ejaculated sperm from men with oligospermia. However, this increased aneuploidy in azoospermic men cannot

easily explain the dramatic increase in chaotic chromosomal abnormalities of TESE embryos. That's why we *suspect* that other sperm abnormalities are the cause, like the centrosome.

It's known that aneuploidy of embryos is not closely linked or correlated with embryo morphology. As women age and the rates of aneuploidy rise, abnormalities in embryo morphology don't increase.<sup>62</sup> However, mosaicism, chaotic mosaicism, and polyploidy are associated with more morphologic embryo abnormalities and do not increase with age. Aneuploidy appears to increase with maternal age and is related to defects in the egg, but mosaicism and chaotic mosaicism may be more related to sperm defects and may result in a high percentage of chaotic mosaic embryos derived from ICSI with nonobstructive azoospermia. Because the spermatozoon centrosome controls the first mitotic divisions, there may be abnormal chromosome distribution among sister cells.<sup>76</sup>

Severe spermatogenic defects, as in nonobstructive azoospermia, may result in higher rates of mosaic and chaotic mosaic embryos, resulting in less efficient implantation, but delivered pregnancy rates can still be very high if either preimplantation genetic diagnosis or other methods of embryo selection are used to pick out the normal embryos from the larger number of abnormal ones. Correspondingly, the live birth rate for TESE with nonobstructive azoospermia is somewhat lower

than for obstructive azoospermia (normal spermatogenesis).

Thus, despite the dramatic success of TESE-ICSI for azoospermic men with severe spermatogenic defects, in these severe cases, post-meiotic sperm factors adversely impact results. However, the negative impact of sperm factors is only a modest one (causing mosaicism), compared to the negative impact of a wife's age (causing aneuploidy). The good news, then, is that most men with a severe spermatogenic defect causing azoospermia can have healthy children using TESE and ICSI. However, the success rate is somewhat reduced compared to ICSI for oligospermia.

## Conclusions

Defects in spermatogenesis resulting in oligoasthenospermia are for the most part genetic in origin and not amenable to correction with any current treatment methods other than ICSI. Severe defects such as azoospermia are caused by new mutations (to which humans are very prone), and milder spermatogenic defects are usually inherited from parents. Male-factor infertility is thus likely to be transmitted to ICSI offspring, but this has not dissuaded infertile couples with male factor from seeking their own genetic child. However, please note that obstructive azoospermia caused by CF gene mutations will only be transmitted to the next generation if the offspring's spouse also has a CF mutation (5%). ICSI with or without testicular or epididymal sperm retrieval can thus allow the vast majority of couples



with severe male infertility to now have their own genetic child, and the only negative consequence is the transmission of male infertility to the next generation. □

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