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Does intracytoplasmic sperm injection lead to a rise in the frequency of microdeletions in the *AZFc* region of the Y chromosome in future generations?

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Microdeletions in the *AZFc* region of the Y chromosome are found in oligo- and azoospermic men. These mutations were genetically lethal before the intracytoplasmic sperm injection (ICSI) era but they can nowadays be transmitted to next generations via ICSI. We have tried to answer the question, 'Does ICSI lead to a significant rise in the frequency of these microdeletions in future generations?', by developing a mathematical model for Y-linked mutations with two variables (fitness and mutation frequency). To illustrate this model we have made estimates according to three imaginary scenarios. Using the assumptions described, the model predicted that the frequency of microdeletions in the *AZFc* region would increase in each generation until a plateau was reached. The higher the fitness, the higher the plateau and the later the plateau would be reached. Taking realistic estimates for fitness (0.5) and spontaneous mutation frequency (0.0001), the maximum increase in men with microdeletions would be twofold. This maximum would be already reached after five generations. However, if the fitness of these men were improved and approached 1.0, the mechanism of selection would disappear and finally all men would have the deletion in the *AZFc* region. Because of the assumptions in these scenarios, these estimates have limitations. The model presented shows that the rise in the frequency of men with microdeletions in the *AZFc* region in future generations would be limited as long as the fitness of these men remained limited.

Key words: AZF/future/ICSI/microdeletion/Y chromosome

Introduction

Intracytoplasmic sperm injection (ICSI) may lead to an increase in infertile men in future generations, since we know that genetic factors play a role in male infertility. However, there are no data available on the impact of ICSI on the transmission of these genetic factors to future generations.

ICSI has caused a revolution in the treatment of severe male infertility (Van Steirteghem *et al*., 1993). The direct injection of a single spermatozoon into the oocyte enables pregnancies in couples where the man has very low sperm counts. Even in azoospermic men, it is possible to perform ICSI by using spermatozoa obtained from the epididymis (micro-epididymal sperm aspiration) (Tournaye *et al*., 1994) or testis (testicular sperm extraction) (Silber *et al*., 1995). Unfortunately, this therapeutic revolution has not been accompanied by a revolution in aetiology and, in most cases, we do not know the underlying cause of male infertility (Bhasin *et al*., 1994).

Currently, there are indications that genetic factors play an important role in male infertility, and the possible transmission of these genetic factors to the offspring via ICSI is a major concern (Engel *et al*., 1996). Recently, microdeletions in the *AZFc* region of the Y chromosome have been described in men with severe oligo- and azoospermia (Reijo *et al*., 1995; Vogt *et al*., 1996; Girardi *et al*., 1997; Kremer *et al*., 1997; Pryor *et al*., 1997). These de-novo mutations create a unique situation from a population–genetic point of view: firstly, they are Y-linked and thus limited to the male population; and secondly, they were genetically lethal before the ICSI era but can nowadays be transmitted to next generations via ICSI.

The main question of this paper is: 'Does ICSI lead to a significant rise in the frequency of microdeletions in the *AZFc* region in future generations?'. We have tried to answer this question by developing a mathematical model for Y-linked mutations with two major variables (spontaneous mutation frequency and fitness). To illustrate this model we have tried to make estimates for both variables, according to three imaginary scenarios.

Materials and methods

Mathematical model

The first step was to develop a mathematical model for Y-linked mutations with the two variables fitness and spontaneous mutation frequency. The fitness (f) or the relative fertility of men with microdeletions in the *AZFc* region is the quotient of the number of children per man with a microdeletion in that region, and the number of children per man in the general population. The spontaneous mutation frequency (μ) is the frequency of men with de-novo microdeletions in the *AZFc* region.

A specific generation (n) consists of p_n men with the wild-type allele (no microdeletion) and q_n men with the mutant allele (microdeletion in the *AZFc* region). The p_n men are expected to father $x_n{*}p_n$ sons, where the factor x_n represents the number of sons per man in the general population of the n^{th} generation. Of these sons, $\mu{*}x_n{*}p_n$ have the mutant allele and $(1-\mu){*}x_n{*}p_n$ have the wild-type allele. The q_n men with a mutant allele are expected to father $f{*}x_n{*}q_n$ sons, and all of them have the mutant allele.

The number of men with the wild-type allele in the $n+1^{th}$ generation

(p_{n+1}) is $(1–\mu)*x_{n*}p_n$. The number of men with the mutant allele in the $n+1^{th}$ generation (q_{n+1}) is $(\mu*x_{n*}p_n)$ plus $(f*x_{n*}q_n)$. In matrix notation:

$$\begin{pmatrix} p_{n+1} \\ q_{n+1} \end{pmatrix} = x_n \begin{pmatrix} 1–\mu & 0 \\ \mu & f \end{pmatrix} \begin{pmatrix} p_n \\ q_n \end{pmatrix} \cong x_n \begin{pmatrix} 1 & 0 \\ \mu & f \end{pmatrix} \begin{pmatrix} p_n \\ q_n \end{pmatrix}$$

The latter approximation was based on the fact that μ is small compared with 1 and 1-*f*. Using matrix calculus it is now possible to calculate $q_{(n)}$, the frequency of the mutant allele in the n^{th} generation:

$$q_{(n)} = \frac{\mu(1 - f^{n+1})}{\mu(1 - f^{n+1}) + (1 - f)}$$

After infinite number of generations:

$$q_{(\infty)} = \frac{\mu}{\mu + (1 - f)}$$

Estimates of the fitness

The second step was to make estimates of the fitness of men with microdeletions in the *AZFc* region. Estimating the fitness was not easy, since the final figure depended on a number of uncertain data. We have tried to approach this problem by making calculations according to three imaginary scenarios. Assuming that ICSI is the only way for men with microdeletions in the *AZFc* region to father children, estimating the number of children of these men depends on four items: the percentage of the couples who choose ICSI; the cumulative live-birth rate after ICSI; the multiple pregnancy rate after ICSI; and the percentage of couples who go for a second or third child after a successful ICSI treatment.

The percentage of men with microdeletions who chose ICSI

Some of the men with microdeletions never visit a fertility clinic and, after genetic counselling, some of them choose alternatives such as donor insemination or adoption. There are no data available on this topic. In our three scenarios we chose more or less arbitrary percentages of 50%, 75% or 100%, respectively.

The cumulative live-birth rate after ICSI

In our centre this rate was 41% after a maximum of five cycles (unpublished data, 1994). The literature provides data only on the cumulative live-birth rate after in-vitro fertilization (Tan *et al*., 1992; Bergh *et al*., 1995; Stolwijk *et al*., 1996). In the three scenarios we chose cumulative live-birth rates of 45%, 60% or 75%, respectively.

The multiple pregnancy rate

In a Dutch study the multiple pregnancy rate was 22%, if two embryos were transferred, and 30%, if three embryos were transferred (Roest *et al*., 1997). In the three scenarios we chose a twin rate frequency of 10%, 20% and 30%, respectively. We did not consider triplets because they hardly contribute to the total number of children after ICSI.

The percentage of couples who go for a second or third attempt after an earlier successful ICSI treatment

This decision depends on a number of social and cultural factors. There are no data available. In the three scenarios we assumed that 60%, 80% or 100% of the couples would go for a second attempt, if they had one child after the first ICSI treatment, and that 0%, 10% or 20% would go for a next attempt, if they had two children after previous treatment(s).

Table I shows the calculations necessary to estimate the total number of children per ICSI man. The product of this estimate and the percentage of men with microdeletions who choose ICSI is the estimate of the number of children per man with these microdeletions.

Table I. Estimate of the total number of children per man undergoing intracytoplasmic sperm injection (ICSI)

Options			Number of children
Single birth	1st treatment		a(1–b)
Twin birth	1st treatment		2ab
Single birth	2nd treatment	Earlier single birth	ca(1–b) a(1–b)
Twin birth	2nd treatment	Earlier single birth	ca(1–b) 2ab
Single birth	2nd treatment	Earlier twin birth	dab a(1–b)
Twin birth	2nd treatment	Earlier twin birth	dab 2ab
Single birth	3rd treatment	2 earlier single births	eca(1–b) a(1–b) a(1–b)
Twin birth	3rd treatment	2 earlier single births	eca(1–b) a(1–b) 2ab

a = cumulative live-birth rate per treatment programme; b = % twins; c = % couples with one child after the 1st treatment who go for a 2nd treatment; d = % couples with a twin after the 1st treatment who go for a 2nd treatment; e = % couples with single births after the 1st and 2nd treatment who go for a 3rd treatment.

Table II. Estimates of the fitness of men with microdeletions according to three scenarios

	Scenario 1	Scenario 2	Scenario 3
Men with microdeletions choosing ICSI (%)	50	75	100
a	45	60	75
b	10	20	30
c	60	80	100
d	0	10	20
e	0	10	20
n children per ICSI man (see Table I)	0.62	1.02	1.58
n children per man with microdeletion	0.31	0.76	1.58
n children per normal man	1.53	1.53	1.53
Fitness	0.2	0.5	1.0

a = cumulative live-birth rate per treatment programme; b = % twins; c = % couples with one child after the 1st treatment who go for a 2nd treatment; d = % couples with a twin after the 1st treatment who go for a 2nd treatment; e = % couples with single births after the 1st and 2nd treatment who go for a 3rd treatment.
ICSI = intracytoplasmic sperm injection.

To calculate the fitness we needed to know the mean number of children per man in the general population. In The Netherlands, the 'Centraal Bureau voor Statistiek' presents yearly demographic data. In 1996, the mean number of children per Dutch woman (the sum of age-specific numbers) was 1.53 (http://www.cbs.nl). Since there were no data on Dutch men, we took this second-best figure.

Table II shows the three imaginary scenarios with their assumptions and the final estimates of the fitness of men with microdeletions in the *AZFc* region. The estimates of the fitness range from 0.2 (scenario 1), 0.5 (scenario 2) to 1.0 (scenario 3).

Estimates of the spontaneous mutation frequency

To estimate the spontaneous mutation frequency in the general population, we needed to know the frequency of microdeletions in the *AZFc* region of men with severe oligo- or azoospermia and the prevalence of severe oligozoospermia in the general population.

The frequency of microdeletions in the *AZFc* region of men with severe oligozoospermia ranges from 2 to 10% (Reijo *et al*., 1995; Vogt *et al*, 1996; Kremer *et al*., 1997; Pryor *et al*., 1997). The prevalence of severe oligo- or azoospermia is not known exactly, but can be estimated by multiplying the prevalence of severe oligozoospermia in infertile couples by the prevalence of infertility in the general population. The prevalence of severe oligozoospermia in an infertile population is about 3% (Hargreave, 1990) and the prevalence

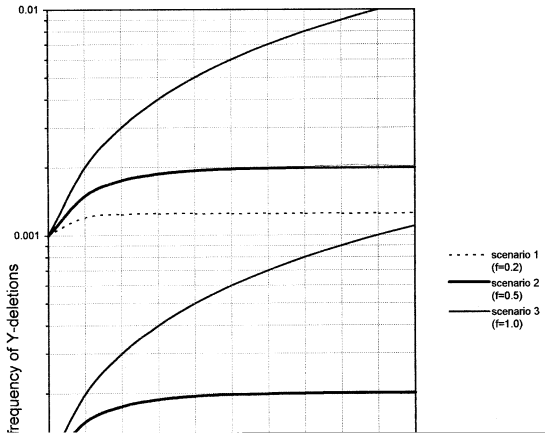

Figure 1. The estimated frequency of microdeletions in the *AZFc* region in the next 10 generations, according to three mutation frequencies [$\mu = 0.001$ (upper graph), $\mu = 0.0001$ (middle graph) and $\mu = 0.00001$ (lower graph)] and three fitness scenarios [$f = 0.2$ (dotted line), $f = 0.5$ (thick line) and $f = 1.0$ (thin line)].

of infertility in the general population is 10 to 15% (Thonneau *et al.*, 1991).

Now it is possible to estimate the spontaneous mutation frequency: (2 to 10%)(10 to 15%)(3%) = 0.6 to 4.5 $\times$ 10^{-4}. According to this estimate, about one in 10 000 men will have a spontaneous microdeletion in the *AZFc* region. To illustrate our model, we chose three mutation frequencies: 0.001, 0.0001 and 0.00001.

Results

Figure 1 shows the estimated frequency in the next 10 generations according to the three fitness scenarios ($f = 0.2$; $f = 0.5$; $f = 1.0$) and three mutation frequencies ($\mu = 0.001$; $\mu = 0.0001$; $\mu = 0.00001$). The frequency of microdeletions started in generation 0 at the level of the spontaneous mutation frequency and increased in each subsequent generation until a plateau was reached. At that moment an equilibrium existed between inflow of mutations and selection. The generation in which this equilibrium was reached depended on the fitness: the higher the fitness, the later this plateau was reached. Assuming a fitness of 0.2 (scenario 1) and a mutation frequency of 0.0001 (middle curve of the lower graph), a maximum frequency of 0.00012 was reached after about two generations. Assuming a fitness of 0.5 (scenario 2) and again a mutation frequency of 0.0001 (middle curve of the middle graph), a maximum frequency of 0.0002 was reached after about five generations. Assuming a fitness of 1.0 (scenario 3) and again a mutation frequency of 0.0001 (middle curve of the upper graph), the frequency was higher, but the maximum frequency was not reached after the 10 generations shown.

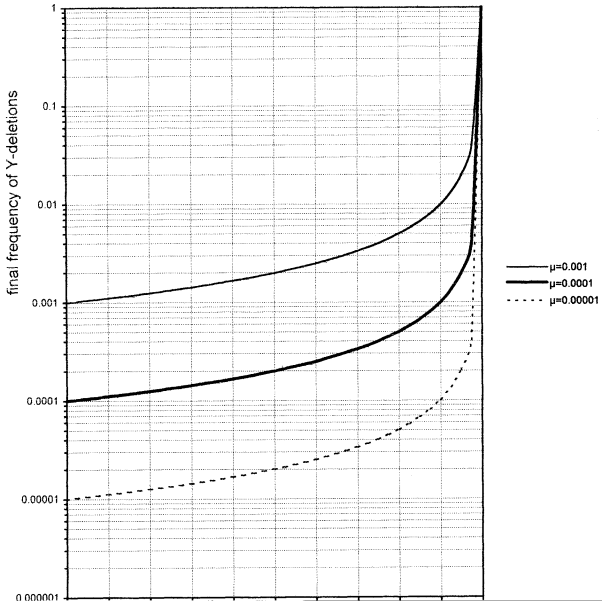

Figure 2. Estimate of the final frequency of microdeletions in the *AZFc* region after infinite generations for three mutation frequencies: $\mu = 0.001$ (thin line), $\mu = 0.0001$ (thick line) and $\mu = 0.00001$ (dotted line).

The mutation frequency has no major influence on the shape of the semi-logarithmic curves. In other words, the relative increase of the frequency was the same for the three mutation frequencies shown. Only the starting point was different.

Figure 2 shows the final frequency of microdeletions after infinite generations. The final frequency rose with increasing fitness, especially if the fitness exceeded 0.9. In scenario 1 ($f = 0.2$) the final frequency of men with microdeletions would be 1.2 times higher than the spontaneous mutation frequency. In scenario 2 ($f = 0.5$) the final increase would be twice as high. If the fitness approached 1.0 (scenario 3), the final frequency of men with microdeletions would go to 100%.

Discussion

The presented model shows that the rise in the frequency of men with microdeletions in the *AZFc* region in future generations is limited as long as the fitness of these men remains limited. Taking the middle scenario for the estimate of the fitness ($f = 0.5$) and for the estimate of the spontaneous mutation frequency ($\mu = 0.0001$), the maximum increase in men with microdeletions is twofold. This maximum is already reached after five generations. However, if the fitness of these men improves and approaches 1.0, the mechanism of selection disappears and finally all men will have the microdeletion, although this will take many generations. In other words, as long as ICSI is not as successful as normal reproduction, the fitness of men with microdeletions remains relatively low, and we should not be too worried about an increase in microdeletions in future generations.

The presented model is unique, since the introduction of ICSI offers the opportunity to study a mutation on the Y chromosome that changes in one generation from a genetically

lethal mutation to a mutation with a certain fitness. The Hardy–Weinberg equilibrium, which states that the relative frequency of a genotype is constant unless there is a change in fitness, is disturbed and a new equilibrium will be installed.

The mathematical model is based on the assumption that ICSI is the only way for men with microdeletions in the *AZFc* region to have offspring. Indeed, these men have severe oligo- or azoospermia (Reijo *et al*., 1995; Vogt *et al*., 1996; Kremer *et al*., 1997, Pryor *et al*., 1997), and therefore spontaneous conception will be rare. However, father/son pairs with the same microdeletion have been described (Vogt *et al*., 1996; Pryor *et al*., 1997). These fathers just may have been 'lucky', although the detected microdeletions also could be polymorphisms without clinical meaning.

The presented mathematical model has some limitations. It is based on the assumption that the mutation frequency is relatively small with regard to 1.0 and to 1.0 minus the fitness, and this is not the case if the fitness approaches 1.0. Furthermore, the predictions for future generations are based on the assumption that the fitness and mutation frequency remain the same in all generations. This will probably not be the case for the fitness, since it might be expected that ICSI and future reproductive techniques will be increasingly successful in the next generations. However, knowing these limitations, the model can be used to give an impression of future developments in the frequency of microdeletions.

The estimate of the fitness has limitations. The absence of adequate data in the literature is a particular problem: the percentage of men with microdeletions who choose ICSI, and the percentage of couples who go for a second attempt after initially successful treatment are unknown. The first percentage is important because it has a major influence on the final estimate of the fitness. The second percentage is of less importance because it hardly influences the final estimate. The lack of adequate data means that making an accurate estimate of the fitness is difficult.

Finally, the estimate of the spontaneous mutation frequency of microdeletions in the *AZFc* region in the general population has limitations, because the available data are based on studies performed in selected populations of infertile men. Extrapolation of these data to the general population is difficult, because different definitions or inclusion criteria might be used. However, our estimate of the spontaneous mutation frequency (0.6 to 4.5 per 10 000 men) is comparable to that of earlier studies (1.0 and 1.1 per 10 000 men) (Reijo *et al*., 1995; Vogt *et al*., 1996). It is important to emphasize that we made the estimates of the fitness and mutation frequency only to illustrate the mathematical model. We did not intend to calculate the exact frequency of microdeletions in future generations, because there were too many uncertain factors to make accurate estimates.

We have developed the present model for microdeletions in the *AZFc* region of the Y chromosome. These deletions will be responsible for only a fraction of all male fertility problems. Therefore our conclusions about the frequency of these microdeletions in future generations cannot be translated automatically into the frequency of all male fertility problems with a genetic basis. However, the general principle that the rise in frequency of mutations is limited, as long as the fitness is limited, keeps its value.

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