deficient[4] and bcl-2 misexpressed (overexpressed)[5] mice accumulate abnormal spermatogonia and harbor degenerate germ cells. There is a well documented relationship between generation of ROS in semen and sperm dysfunction[6], although, ROS generation in the human testis and its resulting affects on spermatogenesis remain to be clearly documented.

Our preliminary work on semen samples from both patients and fertile donors demonstrates a series of ΔmtDNA in spermatozoa which vary in size and ratio when compared to wild type. The intensity of these deletions is related to asthenozoospermia in a small proportion of men. However, it is unlikely that one particular form of ΔmtDNA would be responsible for asthenozoospermia, and thus the accumulated effect of several mutations would seemingly be appropriate[7].

In humans, it is generally accepted that mtDNA is maternally inherited. The oocyte is dormant, at a low metabolic rate, thus preserving its mitochondria. It is then the intact, under-utilized maternal mtDNA that are transmitted while paternal mtDNA are sacrificed[8]. Experimental evidence suggests that low levels of ΔmtDNA are seen in oocytes, though there is an increase of ΔmtDNA with female age[9]. However, aged oocytes, with a fault in the genetic filter, may result in transmission of higher levels of maternal ΔmtDNA. In such cases, we hypothesize that leakage of paternal mtDNA is a possibility. In addition, specifically with Intracytoplasmic sperm injection (ICSI), where immotile spermatozoa are injected into the human egg and achieve fertilization, there may be a greater risk of transmitting defective paternal mtDNA resulting in an increasingly infertile male population and mitochondrial disease.

Interestingly, even when considering *in vivo* conception, an on-going study in our clinic indicates that for women over the age of 40, there is a significantly greater chance of their sons being infertile, due to asthenozoospermia ($P < 0.05$), than for sons of women under 20 (sample size $n = 118$).

With the increasing demand for the use of assisted conception in older women[10] and the widespread use of intracytoplasmic sperm injection (ICSI) with defective spermatozoa, mitochondrial genetic analysis takes on greater significance for infertility.

JUSTIN C. ST. JOHN, IAN D. COOKE & CHRISTOPHER L R BARRATT

University Department of Obstetrics & Gynaecology, Jessop Hospital for Women, Sheffield, 7RE, UK

Johns replies — I appreciate the comments of Drs. St. John, Cooke and Barratt. Based on our experience with a number of different mitochondrial diseases, we concur that defects in mitochondrial DNA (mtDNA) may contribute to defective sperm function and hence to male infertility.

Several different types of mtDNA mutations may be operative in sperm dysfunction. One category may be acquired, somatic mutations, possibly generated via the reactive oxygen species mechanism hypothesized by the authors. Alternatively these mutations may occur throughout the body as part of a multisystemic disorder, as illustrated by the following patient.

A 40-year-old man had a long history of progressive gait ataxia, dysarthria and chronic progressive external opthalmoplegia. He was among the first series of patients with a novel mitochondrial disease phenotype of sensory ataxic neuropathy, dysarthria and opthalmoplegia (SANDO) (Fadic *et al.*, manuscript in preparation). Each patient had significant proportions of multiple mtDNA deletions in skeletal muscle and this particular patient also had multiple deletions in a sural nerve biopsy. He subsequently developed infertility and a work-up revealed abnormal sperm motility and it is likely that they also harbored similar multiple mtDNA deletions.

Another interesting issue is the potential for paternal transmission of sperm mtDNA. Among mammals, the paternal transmission of mtDNA (albeit a low frequency) was first demonstrated in interspecies crosses of mice[11] and recent work has confirmed the occurrence of paternal transmission of mtDNA in murine interspecies crosses[12]. However, in murine intraspecies crosses, paternal mtDNA is rapidly eliminated during early embryogenesis[12]. Therefore mtDNA does not appear to be paternally transmitted in normal mammalian intraspecific matings. Presumably the paternal mtDNA injected directly into the oocyte via intracytoplasmic sperm injection (ICSI) would be eliminated via a similar mechanism early in embryogenesis, but this has not been studied in humans. The role of mtDNA mutations in human male infertility is obviously a fertile area for further investigation.

DONALD R. JOHNS

Human Mitchondrial DNA Laboratory, Harvard Institutes of Medicine, Room 858, 77 Avenue, Louis Pasteur, Boston, Massachusetts 02115, USA

1. Johns, D.R. The other human genome: Mitochondrial DNA and disease. *Nature Med.* **2**, 1065–1068 (1996).
2. Folgero,T. *et al.* Mitochondrial disease and reduced sperm motility. *Hum. Reprod.* **8**, 1863–1868 (1993).
3. Zamzami, N. *et al.* Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. *J. Exp. Med.* **182**, 367–377 (1995).
4. Knudson, C.M. *et al.* Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* **270**, 96–99 (1995).
5. Furuchi,T. *et al.* Inhibition of testicular germ cell apoptosis and differentiation in mice misexpressing Bcl-2 in spermatogonia. *Development* **122**, 1703–1709 (1996).
6. Aitken, R.J., Irvine D.S. & Wu, F.C. Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. *Am. J. Obstet. Gynecol.* **164**, 542–551 (1991).
7. Ozawa, T. Mechanism of somatic mitochondrial DNA mutations associated with age and diseases. *Biochim. Biophys. Acta.* **1271**, 177–189 (1995).
8. Allen, J.F. Separate sexes and the mitochondrial theory of ageing. *J. Theor. Biol.* **180**, 135–140 (1996).
9. Keefe, D. L. *et al.* Mitochondrial deoxyribonucleic acid deletion in oocytes and reproductive aging women. *Fertil. Steril.* **64**, 577–583 (1995).
10. Human Fertilisation and Embryology Authority, 5th Annual Report (1996).
11. Gyllensten, U., Wharton, D., Josefsson, A. & Wilson, A.C. Paternal inheritance of mitochondrial DNA in mice. *Nature* **353**, 255–257 (1991).
12. Kaneda, H. *et al.* Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. *Proc. Nat. Acad. Sci. USA* **92**, 4542–4546 (1995).

One too many tricks

To the editor — Your news item "Choosing a polio vaccine is tricky business" (*Nature Medicine* **3**, 7; 1997) contains a major error. In referring to the IPV, the article states: "the killed virus, injected intravenously . . . " The IPV is injected intramuscularly. An intravenous injection could well be lethal. The tricky business should not be made trickier.

MICHAEL KATZ

The March of Dimes Birth Defects Foundation 1275 Mamaroneck Avenue White Plains, New York 10605, USA