piece could be observed and the beginning tail was very rigid when touched. Due to the narrow diameter of the injection pipette (inner diameter 6 μm) compared to that of the spermatid, we aspirated it very slowly. During this step, the cell was compressed and deformed and took an elongated 'cigar-like' shape inside the lumen of the pipette.

After injection into the cytoplasm, it was difficult to see whether the spermatid membrane had retained its total integrity, since immediately after the procedure it disappeared into the ooplasm. When it came out of the pipette, it probably swelled again to recover its initial shape, and the spermatid plasma membrane broke. Presumably the nucleus was very contracted, as it could not be seen.

Twenty hours after the micro-injection, three eggs with a second polar body showed two pronuclei. The presence of two distinct pronuclei and two polar bodies was also observed in the egg injected with the spermatid. All oocytes cleaved further to 4-cell embryos of grade B quality.

Discussion

Our results indicate that a late-stage spermatid was able to activate a human egg. It is premature to announce that oocytes micro-injected with spermatids will fertilize as well as those injected with testicular spermatozoa, since with the latter our pregnancy success rate is 28%. Nevertheless, this is an encouraging step for an important group of men since a blockage of spermatogenesis at the spermatid level is a frequent occurrence. The whole approach also rekindles interest in testicular biopsies.

Even in cases where FSH concentrations are (moderately) elevated, quantitative study of spermatogenesis shows a certain amount of spermatozoa and an eventual block of spermiogenesis, i.e. at the spermatid level. Oocyte fertilization by a spermatid offers new possibilities for treatment of this type of andrological deficiency.

References

Mallidis, C. and Baker, G. (1994) Fine needle tissue aspiration biopsy of the testis. *Fertil. Steril.*, **61,** 367–375.

Nijs, M., Geerts, I., van Roosendaal, E., Segal-Bertin, G., Vanderzwalmen, P. and Schoysman, R. (1993) Prevention of multiple pregnancies in an in vitro fertilization program. *Fertil. Steril.*, **59,** 1245–1250.

Ogura, A. and Yanagimachi, R. (1993) Round spermatid nuclei injected into hamster oocytes from pronuclei and participate in syngamy. *Biol. Reprod.*, **48,** 219–225.

Ogura, A., Yanagimachi, R. and Usui, N. (1993) Behaviour of hamster and mouse round spermatid nuclei incorporated into mature oocytes by electrofusion. *Zygote*, **1,** 1–8.

Ogura, A., Matsuda, J. and Yanagimachi, R. (1994) Birth of normal young after electrofusion of mouse oocytes with round spermatids. *Proc. Natl. Acad. Sci. USA*, **91,** 7460–7462.

Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, **340,** 17.

Schoysman, R., Vanderzwalmen, P., Nijs, M., Segal-Bertin, G. and van de Casseye, M. (1993a) Successful fertilization by testicular spermatozoa in an in-vitro fertilization programme. *Hum. Reprod.*, **8,** 1339–1340.

Schoysman, R., Vanderzwalmen, P., Nijs, M., Segal, L., Segal-Bertin, G., Geerts, L., van Roosendaal, E. and Schoysman-Deboeck, A. (1993b) Pregnancy after fertilisation with human testicular spermatozoa. *Lancet*, **342,** 1237.

Sofikitis, N., Zavos, P., Koutselinis, A., Mourtzinis, D., Loutradis, D. and Glanzounis, G. (1994) Achievement of pregnancy after injection of round spermatid (RS) nuclei into rabbit oocytes and embryo transfer: a possible mode of treatment for men with spermatogenic arrest at the spermatid stage. *Eighty-Ninth Annual Meeting of the American Urological Association*, San Francisco, CA, USA. *J. Urol.*, **5** (Suppl.), 151.

Van Steirteghem, A., Liu, J., Joris, H., Nagy, Z., Janssenswillen, C. and Tournaye, H. *et al.* (1993) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod.*, **8,** 1061–1066.

What forms of male infertility are there left to cure?

Sherman J.Silber

St Luke's Hospital, 224 South Woods Mill Road (Chesterfield), St Louis, MO 63017, USA

The development of intracytoplasmic sperm injection (ICSI) has completely reversed the way we look at male factor infertility. There may soon be virtually no form of male infertility, aside from bilateral anorchia, that is not amenable to treatment. Until recently, it was thought that the major stumbling block to treatment of infertile couples was the infertile male. In the near future, the only limitation to treatment may be the female, and more specifically in most cases, simply the age of the female.

With ICSI, the presence of only a few very weak, barely twitching spermatozoa in a semen specimen (i.e. severe oligoasthenozoospermia) is all you need for normal fertilization rates (Van Steirteghem *et al.*, 1993). Men with apparent azoospermia may have a few poor quality spermatozoa noted after centrifugation at 1800 *g*, and these too may be sufficient for normal fertilization and pregnancy. ICSI now even appears to offer a solution for men with absolutely no spermatozoa at all in the centrifuged ejaculate (true azoospermia).

Azoospermia is caused either by obstruction or deficient spermatogenesis. In the case of obstruction, microsurgery gives high success rates, with 88% of cases yielding spermatozoa in the ejaculate that are adequate either for spontaneous pregnancy or at least for ICSI (Silber, 1989). When reconstructive surgery fails, or in cases of irreparable obstruction such as congenital absence of vas deferens (CAV), the prospect for such patients with obstructive azoospermia had been considered in the past to be dismal. Epididymal spermatozoa retrieved from such men are known to yield very low fertilization and pregnancy rates with in-vitro fertilization (IVF). However, it is now clear that microsurgical epididymal sperm aspiration (MESA) combined with ICSI gives pregnancy rates and delivery rates in these cases that are no different from those of men with normal sperm counts (Silber *et al.*, 1994).

In cases of obstructive azoospermia where no motile spermatozoa at all are retrievable from the epididymis, testicular sperm extraction (TESE) combined with ICSI also gives

high fertilization and pregnancy rates (Devroey *et al.*, 1994; Silber *et al.*, 1995). With the use of this technology, even men with no epididymis at all should have no problem in having a child. Thus, all cases of obstructive azoospermia can now be successfully treated.

So what about men with non-obstructive azoospermia caused by deficient spermatogenesis? With the exception of Kallman's syndrome, all efforts to stimulate greater spermatogenesis in such patients using hormones or other treatments, including surgery for varicocele, have failed to demonstrate any effectiveness. Deficient spermatogenesis cannot be stimulated by any of the conventional modes of therapy utilized over the past 40 years. In fact, it is most likely that deficient spermatogenesis is genetically transmitted.

Surely one would think that cases of non-obstructive azoospermia ought to be non-treatable? Such cases would include the presence of Sertoli cell only, maturation arrest, postcryptorchidism tubular atrophy, mumps or Klinefelter syndrome. This would appear to be a fairly hopeless list. Yet we now know that an extremely minute amount of sperm production in a grossly deficient testicle can result in absolute azoospermia in the ejaculate, even though in the testicle there are obviously a few spermatozoa being produced (Silber and Rodriguez-Rigau, 1981). When that is the case, these spermatozoa can be retrieved by TESE, and using ICSI, the fertilization and pregnancy rates obtained may be similar to those of couples in whom the man has a normal sperm count (Devroey *et al.* 1995). Even if examination of a fixed-tissue testicle biopsy slide shows only a single seminiferous tubule is making spermatozoa where all other tubules show zero spermatogenesis, we can be fairly confident of finding enough spermatozoa to do ICSI.

What about the cases where absolutely no spermatozoa are noted in a fixed-tissue testicle biopsy slide? Perhaps surprisingly, a careful 'wet' preparation of a fresh testicular specimen in such cases will often still yield a few spermatozoa. We have achieved pregnancies with couples such as this in whom the male had testicles no bigger than a peanut, along with a very elevated follicle stimulating hormone (FSH) concentration. Neither the FSH concentration, the very small size of the testicle, nor the specific cause of the azoospermia precludes pregnancy using TESE with ICSI. Of course, there certainly will be many cases of non-obstructive azoospermia in which no spermatozoa will be found with TESE, and it is too early to speculate what percentage of these cases of non-obstructive azoospermia will be treatable. But, none the less, it is useful to know that if there is just a single minute focus of sperm production in the testes, despite there being absolutely no spermatozoa in the ejaculate, it may be possible to harvest a few spermatozoa from testicular tissue that can be used for ICSI. Thus far, we have successfully used TESE with azoospermia caused by Sertoli cell only syndrome, maturation arrest, cryptorchid tubular atrophy and anejaculation, as well as obstruction.

One potential problem with small testes is that each TESE procedure will possibly reduce the remaining testicular tissue. A pure speculation is that we might be able to solve this problem by performing a vasectomy. This could allow tiny numbers of spermatozoa to collect in the epididymis, so that they can then be retrieved by MESA rather than TESE.

We can speculate about many other 'hopeless' male factor cases, the treatment of which with TESE and ICSI needs to be studied. For example, we are not aware of any group, including ours, that has achieved pregnancy by injection of completely immotile ejaculated spermatozoa. In most (not all) cases of '100% immotility' in the ejaculate, a careful search will often reveal a few barely twitching spermatozoa. However, in some cases, there truly will not be a single moving spermatozoon anywhere in the specimen. Such spermatozoa (excluding those with Kartagener's syndrome) at present will not give successful results with ICSI. The cause of this phenomenon, excluding Kartagener's, has been known for years often to represent senescent degeneration and perhaps delayed epididymal transport. In these cases, testicular spermatozoa may not be senescent, and lack of motility in these spermatozoa may not be a serious obstacle. Although no pregnancies have yet been achieved using ICSI with non-motile ejaculated or epididymal spermatozoa, one could speculate that pregnancies in such cases may be possible using testicular spermatozoa. One can also speculate that ICSI should be attempted for Kartagener's syndrome, globozoospermia, and even some Klinefelter syndrome cases.

It is probable that most cases of non-obstructive male factor infertility are genetically transmitted. Many of these patients will not get pregnant even with ICSI. Therefore, the fact that there are virtually no 'forms of male infertility that are left to cure' does not at all nullify the strong need for continued intensive research in male infertility. However, this research should be directed at the molecular level, so that perhaps future generations will not require ICSI.

References

Devroey, P., Liu, J., Nagy, Z., Tournaye, H., Silber, S.J. and Van Steirteghem, A.C. (1994) Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection. *Fertil. Steril.*, **62,** 639–641.

Devroey, P., Lui, J., Nagy, Z. Goossens, A., Tournaye, H., Camus, M., Van Steirteghem, A. and Silber, S.J. (1995) Pregnancies after testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia. *Human. Reprod.*, **10**, in press.

Silber, S.J. (1989) Results of microsurgical vasoepididymostomy: role of epididymis in sperm maturation. *Hum. Reprod.*, **4,** 298–303.

Silber, S.J. and Rodriguez-Rigau, L.J. (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.J., Nagy, Z.P., Liu, J., Godoy, H., Devroey, P. and Van Steirteghem, A.C. (1994) Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. *Hum. Reprod.*, **9,** 1705–1709.

Silber, S.J., Van Steirteghem, A.C., Liu, J., Nagy, Z., Tournaye, H. and Devroey, P. (1995) High fertilization and pregnancy rate after intracytoplasmic sperm injection with sperm obtained from testicle biopsy. *Hum. Reprod.*, **10,** 148–152.

Van Steirteghem, A.C., Nagy, Z., Joris, H., Liu, J., Staessen, C., Smitz, J., Wisanto, A. and Devroey, P. (1993) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod.*, **8,** 1061–1066.