Dear Sir,

We want to thank Professor Grudzinskas very much for his comments. We are happy to hear that his experience is similar to ours. Hopefully, the knowledge that the distribution of spermatogenesis in most cases of non-obstructive azoospermia is diffuse will lead to the removal of much smaller amounts of tissue for testicular sperm extraction (TESE), fewer biopsies in these cases, and much reduced risk of testicular damage.

In our paper (Silber et al., 1997), patients had undergone many testicular biopsies on both sides during a single operative procedure until reports came back from the laboratory indicating the presence of spermatoozoa. The fact that such an extensive sampling correlated well (in perhaps 80–90% of cases) with a random prior diagnostic biopsy indicates that in many cases, more tissue was probably removed than was necessary, because it requires so long to do a thorough search in some of these cases. Therefore, the preliminary reports from the laboratory may initially indicate no elongated spermatoozoa, but nonetheless a careful search of the prepared microdroplet from the specimens may very well reveal spermatoozoa. This may especially be true if the tissue is processed with enzyme digestion which we feel gives a more complete release of spermatoozoa from the seminiferous tubules.

The problem that we still face is that in some cases, though the distribution of spermatogenesis is indeed diffuse rather than patchy, nonetheless, it is so sparse that it would still require removal of a large amount of tissue in order to have a chance of finding an occasional tiny spermatogonial focus.

In those difficult cases, another option (rather than removing a large amount of tissue) would be to perform the testicular retrieval completely under microsurgical technique with an operating microscope. It is possible that in those cases of non-obstructive azoospermia in which sperm are not initially retrieved with a very conservative biopsy, whether by needle or by open technique, a microsurgical operation of the testicle may make most apparent those occasional microscopic foci of spermatogenesis without the need to remove a great deal of tissue.

I wish to congratulate Professor Grudzinskas on his work, and appreciate his kind remarks regarding our paper.

References

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Cooling rates in embryo cryopreservation

Dear Sir,
The report from Van den Abbeel et al. (1997) on a randomized comparison of the cryopreservation of 1-cell human embryos using either a controlled-rate procedure or ultra-rapid cooling, was an important contribution to this aspect of assisted reproduction. Their study clearly showed that the percentage of embryos progressing to further cleavage was higher in the slow controlled-rate cryopreserved group, and that there was also a trend (although not significant with the numbers studied) for there to be a higher rate for pregnancies and live births after recovery of the slowly-cooled embryos. These results were obtained in the face of the apparently contradictory data showing that the percentage morphological recovery was significantly higher in the ultra-rapid group, but we agree with the authors’ premise that morphological recovery scored immediately after cryopreservation is a poor prognostic indicator for the true functional recovery of cryopreserved 1-cell embryos or oocytes.

It is likely that the different morphologies of the two groups reflect the different biophysical stresses experienced by the embryos under the two cooling regimes. During controlled rate freezing, exposure to the increasing hypertonic environment of the surrounding medium, as ice slowly grows around the cells, results in embryos assuming a highly-shrunken morphology. Some of these cells will be so damaged during this process that they retain an abnormal morphology upon thawing, which can easily be identified by light microscopy. In contrast, using ultra-rapid techniques, higher concentrations of cryoprotectants are used and the samples plunged directly into liquid nitrogen in an attempt to ‘out-run’ the formation of ice and achieve low temperature storage in a ‘glassy’ or vitreous state. Unfortunately, in practical terms, most biological samples treated this way exist in a metastable state as a mixture of glassy material, small ice crystals and ice nuclei, often too small to be visually detected, but which are capable of growing to form larger ice crystals as water molecules become more mobile during the thawing process (Fahy, 1989). In fact, the faster cooling rates predispose to an increased number (as opposed to size) of these latent ice nuclei. These can unfortunately grow to some extent if there are small fluctuations in temperature during storage (as might be experienced during opening and closing of storage containers) or physical stress on the samples, which can generate cracks through the brittle glassy matrix and ice crystallization along the fracture planes. The development of small but numerous intracellular ice crystals by these mechanisms can result in embryos that have been lethally injured by ‘multiple hits’, but that appear morphologically in good condition immediately after thawing and dilution. However, lethal damage usually becomes more obvious with time after restoration in culture at 37°C. Assessment of gross morphology after a relatively short time (in our experience on oocyte cryopreservation, within 1 h; Hunter et al., 1995) would more closely correlate with the number of truly viable embryos. Ultra-rapid cooling has been shown to be a successful method for preserving 1-cell animal embryos under laboratory conditions. However, it is not necessarily a ‘user-friendly’ robust method which can be readily transferred to the routine clinical setting without paying considerable attention to all of the small technical problems involved with this method.
details which eventually dictate whether viable embryos can be recovered, irrespective of their post-thaw morphology, as borne out by the results reported by Van den Abbeel et al. (1997).

One point which the authors’ make, and which deserves further comment, is the question of whether 1-cell human embryos have an unusual permeability to water or cryoprotectants which might affect their recovery from cryopreservation, but which is currently unknown. Such cell characteristics ultimately dictate what rates of cooling or exposure to cryoprotectants can be safely used, and can be combined to allow computer modelling of optimal cryopreservation methods in models such as those proposed by Karlsson et al. (1996). In fact there are some measurements on water permeability in human 1-cell embryos reported in a thesis study by Hunter (1991). The values obtained for water permeability (0.55 μm/min atmosphere at 20°C and 0.34 μm/min atmosphere at 10°C) are very similar to those for the unfertilized human oocyte (0.44 and 0.31 μm/min atmosphere respectively) or mouse oocyte (0.48 and 0.31 μm/min atmosphere), and suggest that the process of fertilization and incorporation of the male genetic material did not cause a significant alteration in cell membrane characteristics. This is in agreement with earlier studies in murine oocytes and 1-cell embryos, which also showed no change in water permeability (Leibo, 1980); however, differences in permeability to cryoprotectants have been shown (Jackowski et al., 1980). The higher sensitivity of the human 1-cell embryos to cryopreservation (even by controlled rate freezing) may be more to do with the larger overall cell size than in the mouse, requiring better control of conditions during the early phases of ice formation (at the seeding temperature or from seeding to around –60°C) to allow sufficient time for dehydration and to avoid intracellular ice formation. Prospective studies such as those reported by Van den Abbeel et al. (1997), plus additional computer modelling, should improve the efficiency and usefulness of embryo cryopreservation in the clinic.

References

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Dear Sir,
We thank Paynter et al. for their remarks formulated in their Letter to the Editor relating to our paper (Van den Abbeel et al., 1997) on the cryopreservation of 1-cell embryos.

Regarding the morphological survival after thawing we noticed that ultra rapidly frozen–thawed 1-cell embryos that were morphologically intact immediately after thawing remained intact even after 24 h. Their developmental capacity, however, was impaired. The different behaviour of 1-cell embryos in comparison with ultra-rapidly frozen metaphase II oocytes may be explained by a different cytoskeletal organization.

Regarding the permeability characteristics of human embryos for water and cryoprotectants, we support the idea that for optimal vitrification of human embryos, permeability characteristics for water and cryoprotectants are indeed important. Successful vitrification depends on a correct interplay between permeation of cryoprotectants and dehydration. The determination of some measurable biophysical characteristics of human embryos will be important to develop optimal conditions for exposure to the cryoprotectants and vitrification solution. Furthermore, the determination of such biophysical characteristics can be useful when developing optimized protocols for slow-controlled rate freezing using theoretical models.

Clinical andrology today
Dear Sir,
Having largely initiated the recent debate on the training of clinical andrologists (Jequier, 1993; Cummins and Jequier, 1994; Jequier and Cummins, 1997), there are one or two comments I would like to make about the two contributions

References

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