

meeting report

ART: Boon or bane?

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In 1978, the birth of the first baby through *in vitro* fertilization resulted in an explosion of new techniques that are now widely used in reproductive medicine and biology around the world. With these advances have also come many concerns for the safety of these techniques. In addition, even newer technologies are on the horizon, such as cloning and the production/use of stem cells. The 18th annual meeting of the European Society of Human Reproduction and Embryology (ESHRE), held in Vienna from 30 June to 3 July 2002, provided a forum for discussion of these and other relevant topics.

Given that assisted reproduction technology (ART) is a constant feature in current news items, it is important to keep abreast of current developments. Europe leads the world in ART, performing over half of all ART treatment cycles worldwide. In 1998, over 232,000 cycles were reported in 18 European countries¹, resulting in the birth of approximately 40,000 children; more than half of all these cycles were carried out in France, Germany and the United Kingdom. By comparison, approximately 73,000 cycles were reported in the USA and Canada in 1997. In Europe, slightly over half of the cycles utilized standard *in vitro* fertilization (IVF) techniques, with just under half (~46%) of cycles utilizing intracytoplasmic sperm injection (ICSI). We have come a long way since the birth in 1978 of Louise Brown, the first baby born through IVF.

ESHRE was founded to stimulate study in the field of reproductive medicine and science. Membership has risen from approximately 250 in 1985 to over 4,000 scientists, clinicians and paramedics in 2002. Although ESHRE is the leading European society in the area of reproductive science/medicine, more than a quarter of its current members actually come from outside Europe, making it one of the major societies in this area worldwide. The 18th annual meeting of ESHRE attended by more than 4,000 registrants from a total of 91 countries, giving it a truly international flavour.



ESHRE annual meetings always begin with two keynote lectures on topics of general interest to a wide audience. This year, the first lecture was an extremely interesting presentation by Professor H. Seidler (Institute for Anthropology, University of Vienna, Austria). Professor Seidler discussed 'The Iceman', the frozen body discovered in the Alps in 1991, and the subsequent unfolding interpretation of how/why he came to be where he was found. This was followed by Professor R. G. Edwards (formerly in the Department of Physiology, University of Cambridge, UK), a 'founding father' of ART, as well as a founding member of ESHRE. Professor Edwards discussed "The ancient and

modern world of IVF", in which he traced the important discoveries that contributed to the achievement of successful human IVF. This lecture put the relatively new area of ART into a historical context. This was particularly useful for younger scientists and clinicians in the audience, as many probably take the techniques of ART for granted. The breadth of topics covered at the meeting precludes consideration of all talks, so a selection, chosen to include those commonly referred to in the news, will be discussed in some detail.

Multiple pregnancies

In recent years, attention has focused on the worryingly high incidence of multiple

pregnancies resulting from ART. In the early days, multiple embryos (often more than three) were transferred to try to achieve a pregnancy. At that time, relatively little consideration was given to the consequences of having twin or higher order multiple pregnancies. Indeed, couples and clinicians alike often viewed this as an efficient way to get a complete family with one pregnancy. However, such pregnancies, even with twins, put both mother and babies at risk, tie up special care facilities in hospitals and are very costly to society². Consequently, more and more clinics throughout Europe are reducing the number of embryos transferred at any one time, from three or more down to two or even one. In the UK, the Human Fertilisation and Embryology Act restricts the number of embryos used in each attempt to three, but the Human Fertilisation and Embryology Authority guidelines now stipulate that no more than two embryos should be replaced in all but exceptional cases.

Dr K. Nygren (Sofiahmet Hospital, Stockholm, Sweden) and Dr A. N. Andersen (Copenhagen University Hospital, Denmark) presented the latest report from ESHRE's European IVF monitoring (EIM) programme, with data covering 1999–2000. Consistent with the EIM report for the preceding year¹, there continues to be a decrease in both the numbers of embryos transferred and the incidence of multiple pregnancies, but there is still much room for improvement. In particular, there are still several countries where transfer of three or more embryos occurs in more than half of all cycles. Despite this, some of those countries had low pregnancy rates, highlighting a need to improve techniques so as to produce high quality embryos. Progress here requires better conditions for culturing and better criteria for choosing 'good' embryos. Considerable research is being undertaken to identify embryos most likely to implant and to develop to term. A number of presentations in Vienna addressed this need.

The best way to radically reduce multiple pregnancies is to transfer only one

embryo at a time. Although many clinics do not feel that their results are good enough to do this. Dr M. Lukassen and colleagues (University Medical Centre, Nijmegen, The Netherlands) reported results of a preliminary study, in which they found no statistical difference in the success rates obtained after two IVF cycles with a single embryo transfer in each versus one IVF cycle with two embryos being replaced. If it becomes possible to get consistently good results with single embryos, then it should be possible to reduce the hormonal stimulation currently used to ensure that many oocytes will be obtained. This is particularly important, as hormonal stimulation often has undesirable effects on the endometrium that may interfere with successful implantation.

Given the many risks associated with multiple pregnancies, there was much concern about proposals currently being considered by the Italian parliament, which would permit the freezing of oocytes and sperm, but not of embryos. This is despite the fact that freezing of embryos is the most effective means of preserving surplus embryos and oocyte freezing has yet to be proven safe and equally effective. The Italian proposals would only allow a maximum of three oocytes to be inseminated and would require that all resulting embryos be transferred, thus risking multiple pregnancies in many women. Given the strong movement to reduce multiple pregnancies, these proposals were seen as a retrograde step, leading ESHRE to issue a formal statement opposing such changes.

Safety

One question that recurs frequently concerns the safety of ART. Earlier this year, an Australian study³ reported that major birth defects resulting from IVF and ICSI were twice as prevalent when compared with normally conceived children, raising concerns around the world. Dr U-B. Wennerholm and Professor C. Bergh (Sahlgrenska University Hospital, Göteborg, Sweden) were asked by ESHRE to review the evidence in the literature

and to discuss the risks of ART during a press briefing in Vienna. In the Australian study, the sample size of IVF (837) and ICSI (301) children was not large. In a Belgian study⁴ published at approximately the same time, there were similar rates of major malformations in the ~3,000 ICSI and ~3,000 IVF children evaluated. Although there was not a control group of naturally conceived children, the incidence of malformations in the ART children was similar to that found in the Australian control group. After looking at all the published data, Dr Wennerholm and Professor Bergh concluded that there is a small and statistically significant increase in sex chromosome and autosomal anomalies in ICSI children when compared with normally conceived children. There is also a slightly higher risk that ICSI children will have hypospadias, a condition involving malformation of the penis that may be related to paternal subfertility. However, the absolute risk for a serious congenital malformation or chromosomal abnormality after IVF and ICSI seems to be small, a level of risk that many couples who would otherwise remain childless may consider to be acceptable.

ICSI is usually carried out when the numbers and quality of sperm available are low. It is known that reduced and/or defective spermatogenesis is often caused by genetic defects, particularly microdeletions on the Y chromosome; using sperm from such men will pass on the defective Y chromosome to any sons, with consequent effects on fertility. Recent data have suggested that Y chromosomes with a deletion in the azoospermia factor (AZFc) region may be associated with sex chromosome mosaicism, resulting in Turner's syndrome and/or sexual ambiguities because of an absence of a Y chromosome in some of the cells. Dr P. Patsalis and colleagues (Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus) had examined this hypothesis by evaluating a small group of patients that were known to have these phenotypes and a 45,X/46,XY karyotype, to determine whether there were microdeletions on the Y chromosome. In

a third of the patients examined, they found a complete deletion of the AZFc region. These results suggest that when sperm from men with microdeletions in the Y chromosome are used for ICSI, the resulting male children would not only have fertility problems because of the defective Y, but might also have an increased risk of sex chromosome aneuploidy. This would result in sexual ambiguities and conditions such as Turner's syndrome.

Other concerns about long-term safety arose a few years ago when a report from Australia by Dr G. Leslie (Sydney University Department of Obstetrics and Gynaecology, Royal North Shore Hospital, Australia) suggested that ICSI children at the age of one seemed to be at an increased risk of delayed mental development when compared with other children. These findings made it even more important to continue to look at cognitive development as the children got older. The same Australian team reassessed development in the same ICSI, IVF and control children at the age of five, and also in additional ICSI and control children. They found no significant differences in the scores achieved on IQ tests (verbal, performance and full-scale) among the ICSI, IVF and control children, suggesting that ICSI children are not at increased risk for subnormal long-term mental development. This should provide reassurance that ART children will develop normally, but clearly there is a need to continue observing such children until they reach adulthood, in case there might be some relatively late-onset problems.

New applications of ART

Dr C. Rubio (Instituto Valenciano de Infertilidad, Valencia, Spain) reported that previously unrecognized chromosomal abnormalities in embryos seem to be important causes of unexplained recurrent miscarriage and implantation failure. The team carried out preimplantation genetic diagnosis (PGD) in couples where the woman had experienced at least two unexplained miscarriages and in couples with three or more unsuccessful IVF or

ICSI treatments in the previous four years. Results demonstrated that there were significantly more abnormal embryos in these groups of women, compared with those from a control group of fertile women undergoing PGD for sex-linked diseases. The differences were particularly striking in women under the age of 37, in whom age is not a risk factor for chromosomal abnormalities. When only chromosomally normal embryos were transferred, similar high pregnancy rates and low to normal miscarriage rates were obtained in all groups. These results are very encouraging and suggest that ART can be of value in even more couples than previously imagined.

Stem cells

This is a topical and contentious area and one session with invited speakers was devoted to the consideration of stem cells of various types, including those from human preimplantation embryos (embryonic stem cells) and adult stem cells, and their potential uses. Work has already been done on embryonic stem cells derived from 'spare' human embryos; if such cells were to be used for therapeutic treatment, then there would be genetic differences between the donor stem cells and the recipient. An alternative approach is to use somatic cell nuclei from the person requiring treatment to clone human embryos as a genetically identical source of stem cells for treatment. This is so-called 'therapeutic cloning'. Very recently, it has become clear that many adult tissues and organs have stem cells. Furthermore, many of these stem cells have greater plasticity in terms of potential fate than had been previously thought. There seems to be considerable potential for using such stem cells in therapeutic ways for the treatment of major diseases. Because these cells would be taken from the patient and would not involve cloning a human embryo, this approach is more acceptable to many individuals than therapeutic cloning. However, it is not yet clear whether this approach would be suitable for all potentially treatable diseases. For the record, in 1999, ESHRE

took a consensus decision to impose a voluntary moratorium on 'reproductive cloning' and reiterated this stance in 2001. However, ESHRE supports research into cloning for therapeutic purposes.

Other factors

Occasionally, questions are raised as to whether particular lifestyle factors might affect fertility, either *in vivo* or *in vitro*. Dr M. Zitzmann (Institute of Reproductive Medicine, Münster, Germany) presented research that revealed a significant link between smoking, particularly in the male partner, and reduced success in IVF and ICSI. In couples receiving ICSI treatment, smoking was the only significant predictor of outcome. In IVF treatments, other factors — including the number of embryos transferred and female age — also contributed to failure. Smoking probably has a negative effect on fertilization *per se*, but it can also damage DNA in the sperm, resulting in defective embryonic development. The research team concluded that quitting smoking would help increase the chances of success in both IVF and ICSI, although they had no information on how long smokers should have stopped smoking before trying to father children.

Prof. L. Fraser (Kings College, London, UK) reported the first experimental evidence that both naturally occurring and environmental oestrogens significantly affect mouse sperm fertility *in vitro*. Surprisingly, the environmental oestrogens, normally very weak in comparison with natural oestrogens, proved to be approximately 1,000 times more potent than a natural oestrogen when used with sperm. All the oestrogenic compounds evaluated stimulated mouse sperm to become fertile more quickly. Although this might seem to be a desirable effect, in fact, the oestrogens continued to stimulate the sperm, causing some of them to effectively 'burn out'. Such sperm cannot fertilize, even if they are still motile; if similar events occurred within the female reproductive tract then fertilization might be less likely. Further investigations are needed to determine, for example, the

effect on sperm when these compounds are used in combination. Particularly for environmental oestrogens, it is quite likely that several may be present in our bodies at the same time, although perhaps individually at very low levels. One question concerns whether these compounds have an additive effect. If so, then there could be even more of an effect on natural fertility. Furthermore, are there other molecules that could prevent the burn out, and thus make the most of the positive responses to oestrogens? If so, then oestrogens could be very good for fertility. Clearly, these are questions need to be addressed.

Finally, Dr E. Larsen and colleagues (Copenhagen University Hospital, Denmark) evaluated reproductive potential in women who had survived acute lymphoblastic leukaemia as children. Some patients who receive chemotherapy in childhood suffer from ovarian failure, and hence infertility, but the women in the study seemed to have normal ovarian function, as evidenced by regular cycles. However, researchers found that these women tended to have smaller ovaries with fewer developing follicles (and hence fewer potential oocytes) in each cycle. They concluded that the chemotherapy had reduced the number of follicles, possibly resulting in a shortened reproductive period in these individuals when com-

pared with the general population. Until now, the team has recommended that all long-term survivors of childhood cancer with evidence of normal ovarian function should try to have their first child before the age of 30, although this recommendation was not founded on any specific scientific evidence. However, given the results of this new study, the team is now considering offering individual fertility counselling to such women at the relatively early age of 18–20, their message being that if the women wish to have children, they should perhaps consider motherhood before pursuing a career.

Summary

The meeting in Vienna was both exciting and stimulating, allowing attendees to learn about and discuss new developments in reproductive medicine and biology. So, ART: boon or bane? On balance, the general feeling at the meeting was that when used correctly, ART has been, and continues to be, a benefit for society. Since 1978, the new techniques have allowed many thousands of couples to have children when otherwise they would have faced a childless future. Indeed, as discussed above, new studies have revealed additional categories of couples who may well benefit from ART in conjunction with PGD. Concerns regarding the safety

of the techniques have been voiced from the beginning and current data indicate that there is a statistically significant higher risk, although rather small, of chromosomal and congenital abnormalities in children conceived through ICSI. Comparisons between IVF/ICSI children and normally conceived children are not straightforward, as there are may be confounding factors associated with infertile couples that are not present in fertile couples. It is reassuring that the limited studies on mental development in ART children suggest that these children fall within the normal range, but clearly, further long-term follow-up is needed. Finally, it is particularly satisfying to note the increasing emphasis on decreasing the number of embryos transferred during fertility treatment and so decreasing multiple pregnancies, with all their attendant risks. In turn, this may well lead to use of more gentle ovarian stimulation protocols that could have fewer negative effects on endometrial development and implantation. On balance, then, ART is a boon, not a bane. □

1. Nygren, K. & Nyboe Andersen, A. *Hum. Reprod.* **16**, 2459–2471 (2001).
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3. Hansen, M., Kurinczuk, J. J., Bower, C. & Webb, S. N. *Engl. J. Med.* **346**, 769–770 (2002).
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