their series occurring before or within six hours after admission. This emphasises the requirement for early recognition and urgent treatment. Indirect laryngoscopy is safe in adults³ and in experienced hands is the diagnostic method of choice.

In children the use of an artificial airway is part of the standard treatment but in adults this is not necessarily so; and opinion differs about the requirement for an artificial airway in all cases.⁵ In roughly half of the cases in adults the patients have severe dyspnoea, and many of these will improve with humidified oxygen and intravenous antibiotics. The remainder may require rapid airway intervention by an experienced anaesthetist and ear, nose, and throat surgeon, with subsequent observation in a high dependency area. It is frequently difficult to pass an endotracheal tube through the distorted supraglottic anatomy and it may be easier to pass a

bronchoscope (case 2), or an urgent tracheostomy may be required. These cases illustrate that adult epiglottitis is a lifethreatening condition and may require rapid airway intervention.

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For Debate . . .

In vitro fertilisation: legislation and problems of research

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The recent publication of a consultation paper by the Department of Health and Social Security about human infertility services and research on embryos¹ indicates that the recommendations of the Warnock report on human fertilisation and embryology may soon form the basis of legislation to regulate research on embryos and in vitro fertilisation in Britain.² It is therefore timely to consider some of the problems that arise from enacting such legislation.

In August 1986 the Infertility (Medical Procedures) Act 1984, based on the recommendations of a committee chaired by Professor Louis Waller,³ was partially proclaimed in the Australian state of Victoria. This act was the first in the world that regulated in vitro fertilisation and research on embryos, and it is important to examine the effects of the legislation and consider any problems it has created so that future legislation elsewhere may be drafted with these in mind.

The intention of both the legislation and the Warnock report was to preserve existing clinical in vitro fertilisation programmes but to control their future direction and development by establishing a monitoring body with discretionary powers over research. For a biomedical scientist working in this research how realistic is this aim? Problems might arise concerning, firstly, the effects of the legislation on current research; secondly, its implications for clinical practice; and, thirdly, its ramifications for future progress. The act is now partially in force in Victoria, as is its monitoring body, the Standing Review and Advisory Committee; this provides the opportunity of examining the possible problems from the scientist's point of view.

Effects on current research

FREEZING OF OVA

Current research in Victoria is already being affected by the act, particularly research concerning the freezing of human ova. This technique was developed in Victoria in 1985,⁴ and its continued development was encouraged by the Waller committee as a possible means of sidestepping the ethical problems of freezing embryos.⁵ This encouragement is also evident in the act: "Nothing in this Act prevents or inhibits . . . the development of techniques for, freezing or otherwise storing ova."

Cryopreservation of ova entails removing the follicular cells surrounding the egg, immersing it in a cryoprotectant, and freezing it in liquid nitrogen at -196°C for storage. At present about three quarters of eggs survive the freeze-thaw process,⁶ but animal studies indicate that the incidence of chromosomal abnormalities in embryos grown after fertilisation of eggs stored in this way is greatly increased.⁷ It is not known if human ova are similarly affected, and scientists believe that the possibility should be investigated before the procedure is made available to patients—a requirement also mentioned in the Warnock report.² To do this some human eggs must be frozen, thawed, and fertilised, so that the incidence of abnormalities in the zygote and early cleavage stages can be estimated.

In accordance with the requirements of the act,³ permission to undertake such research was sought from the Standing Review and Advisory Committee, but it was refused on the grounds that to consider such an experiment was outside the jurisdiction of the committee. The committee also stated that to allow this experiment would be in breach of as yet unpublished provisions of the act that specify that: "Where ova are removed from the body of a woman, a person shall not cause or permit those ova to be fertilised outside the body of the woman except for the purposes of the implantation of embryos derived from those ova in the womb."⁸ The two sections cited seem to be in conflict with each other. Furthermore, the latter section also seems to be in conflict with the traditional development of scientific research; the development of any freezing technique requires subsequent assessment by controlled experiments. Failure to gain such approval from the committee has left the researchers with a dilemma: to test whether it is possible to fertilise the ova after thawing and produce embryos that survive and form normal chromosomes is illegal, but to offer the procedure to women knowing that there may be an increased risk of spontaneous abortions in the first trimester or of the need for a therapeutic abortion after amniocentesis in the second trimester is both unethical and irresponsible.

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The experiment may be considered unnecessary since the birth of "frozen ova twins" elsewhere in Australia,⁸ but this small sample should not be interpreted as showing that the technique is totally free of hazards. The need for thorough testing still remains and seems preferable to assessing the effects of the procedure retrospectively by studying the incidence of abortions or congenital malformations.

If this procedure were tested and available to patients it would be possible to reduce the number of laparoscopies required by women undergoing in vitro fertilisation; at present only half the pregnancies after in vitro fertilisation result from a single laparoscopy, and some patients require as many as four.⁹ Should the first attempt not result in pregnancy the excess ova that had been collected and stored could be used for subsequent attempts, and if the first pregnancy was successful the eggs could be kept in storage for possible future use. Some might argue that convenience alone does not justify proceeding with experiments on this technique, but its flexibility makes it potentially useful for fertile couples who wish to delay pregnancy for health or professional reasons. Ova could be collected and stored until required. In the long term this might result in a decrease in the incidence of abnormalities, such as Down's syndrome, in babies born to women of advanced maternal age or those having treatment such as radiotherapy. Freezing ova has advantages over freezing embryos should the ova never be required; no ethical problems are created by their disposal.

MICROINJECTION OF SPERM

As with most techniques associated with in vitro fertilisation, the freezing of ova concentrates on female infertility, or bypassing the tubal lesions that cause two thirds of the infertility among couples in Australian in vitro fertilisation programmes.⁹ In vitro fertilisation is, however, now also able to provide some hope for the 12% of infertile couples in whom abnormalities of the sperm are the cause of the problem.⁹ When there are few sperm or their motility is subnormal they are unable to penetrate the zona pellucida of an egg to fertilise it. This can be treated by microinjection of a single sperm into an egg. The procedure has been developed in humans (I Kola, personal communication), and like the freezing of ova it is now in need of testing to assess its safety before being offered to patients as an alternative to the use of donor sperm.

Microinjection of a single sperm bypasses any selective effect of the female reproductive tract against unfit sperm, as does in vitro fertilisation itself, but it also bypasses any selective interaction that may occur among sperm. For this reason microinjection could increase the chances of a defective zygote. The genetic complement of a sperm cannot be determined before its microinjection, so it is important that the chances of an abnormal sperm being selected are assessed, together with the likelihood of the procedure damaging the egg and causing abnormalities during early cleavage divisions of the embryo. Without such testing this technique can be evaluated only retrospectively. As with the freezing of ova, clinical use of the procedure without such research may lead to an increased risk of spontaneous abortions in the first trimester and abortions after amniocentesis in the second trimester; such experiments are also probably illegal.

The lack of a definition of "fertilisation" in the act (and in the report on which it is based) gives rise to some ways of assessing the effects of the techniques, at least in part. Fertilisation takes about 24 hours to complete—that is, from the time of penetration of the sperm to the syngamy or union of the male and female pronuclei.¹⁰ If fertilisation is interpreted as meaning only zygote formation that results from syngamy, thereby precluding prior events, there is some scope for investigating the fertilisability of freeze thawed ova, and whether microinjection of sperm damages ova, by studying the stages before zygote formation. No study of the early divisions of the embryo or assessment of its developmental potential would be possible. The legal interpretation of this approach, however, remains unclear because of the lack of a definition of fertilisation. Although an amendment to the act will soon be put before the Victorian parliament, this research has been halted for the time being.

EMBRYO BIOPSY

Other continuing research affected by the legislation that raises even more complex issues is the development of a technique for prenatal diagnosis of chromosomal and genetic abnormalities in the embryo in vitro. The incidence of congenital malformations after in vitro fertilisation is 2.6%, compared with 1.5% in the Australian population.⁹ A technique for detecting abnormalities in embryos before transfer would reduce or eliminate the need for considering abortion at a later stage—a far more difficult decision than whether or not to transfer a particular embryo to a

woman. The proposed procedure, known as "embryo biopsy," entails removing one or two cells from the embryo at the eight cell stage, freezing the remainder, and culturing the cells removed to provide ample material for cytogenetic and biochemical analysis.¹⁰ From these results the decision can be taken whether to implant the embryo. Some of the problems faced here—for example, with regard to storage—have already been considered; additional problems are raised, however, by the need to remove cells from the embryo and grow them in culture. This is technically difficult but if proficiently performed should not affect the viability of the developing embryo: not all blastomeres need be intact for pregnancy to be established.¹¹ The cells removed are outer cells of the eight cell cluster that will eventually form the extraembryonic membranes and not the embryo itself.¹²

In Victoria a distinction may be drawn between biopsy of the embryo and the techniques discussed above because of the role of the embryo in the development of the procedure. In freezing of ova and microinjection of the sperm the embryo is created by the scientist as a result of testing independent properties of the egg, but in biopsy of the embryo the embryo itself is the research subject of an "experimental procedure" as defined in the act: "experimental procedure" means a procedure that involves carrying out research on an embryo of a kind that would cause damage to the embryo, would make the embryo unfit for implantation or would reduce the prospects of a pregnancy resulting from the implantation of the embryo."¹³ It seems, therefore, that the development of the individual techniques needed for biopsy of the embryo cannot continue without transferring the embryo into a womb. This is despite the fact that in the absence of appropriate research scientists cannot assess either the potential for damage to each embryo or any possible reduction in its prospects for being successfully implanted.

The Waller committee report (but not the legislation) made specific provision for excess embryos taken for in vitro fertilisation to be used for research together with those embryos that give "reasonable doubts about their normality" if appropriate consent was obtained from the couple that provided the gametes, although no embryos were to be created solely for research.³ To be able to conduct research only when such embryos are available creates practical difficulties in organising a controlled research programme. In many instances the use of possibly abnormal embryos may be inappropriate; results from experiments to test if a procedure is increasing the incidence of abnormalities become impossible to interpret if the embryo is abnormal.

There is a problem in assigning the label "abnormal" to the embryo. The only guidance in the report for identifying normality is that doubts about the embryo should arise from examination. The aspect of examination that should be used is not specified. How exactly does a scientist decide that a particular embryo can be used for research? Of the morphological features that it is possible to study, the rate of growth in culture may not indicate the possibility of a normal pregnancy resulting after transfer,¹³ and even zygotes resulting from dispermy may subsequently revert to embryos with apparently normal chromosomes.¹⁴ If the recommendations of the Waller committee are to be adopted by the Standing Review and Advisory Committee further clarification of the meaning of "reasonable doubts about their normality" is required so that scientists may undertake research knowing that the embryo is being used legally.

In the case of biopsy of an embryo the question of whether culturing the removed cells is in violation of the legislation also arises. Under the act certain practices are prohibited, one of which is "cloning."¹⁵ Does culture of these cells constitute cloning? The term is not defined in the act and receives only cursory mention in the Waller report, leaving it unclear whether cloning should be interpreted as cell culture per se or refers only to culture methods aimed at producing complete individuals as defined in the Warnock report.⁵

Successful development of techniques of biopsying embryos would benefit not only patients participating in an in vitro fertilisation programme because of infertility but also those fertile couples who at present remain childless because of genetic risk. The embryos of such couples could be formed in vitro and a biopsy performed so that the chromosomal or genetic constitution for the relevant disorder could be determined before transfer was considered. The cytogenetic and molecular genetic techniques needed are nearly all available, and their continued refinement has a potential for influencing the number of therapeutic abortions in the second trimester in couples in which both parents are carriers for common genetic diseases or one member is a translocation carrier with a high risk of producing chromosomally unbalanced gametes.

The future of these techniques in Victoria depends on the discretion of the Standing Review and Advisory Committee. Foremost in its decisions must be the principles set down in the act: "In the exercise of its functions, the Committee—(a) shall have regard to the principle that childless couples should be assisted in fulfilling their desire to have children; (b) shall ensure that the highest regard is given to the principle that human life shall be preserved and protected at all times; and (c) shall have regard to the spirit and intent of the several provisions of this Act."¹⁵ Until each research

programme is considered with these broad and conflicting directives in mind the problems for the scientist will remain unresolved and the future of the research programmes will be in doubt.

Effects on current practice of in vitro fertilisation

The principles of the Standing Review and Advisory Committee are seen by some as controlling the future developments in in vitro fertilisation programmes but not affecting current clinical practice. Despite the achievement of more than 900 live births or pregnancies in Australia, however, these current practices are themselves still largely experimental.⁹ Neither the techniques used for fertilisation nor the subsequent in vitro culture are yet optimal. In aiming for improvement the scientist needs to know whether day to day variations in laboratory procedures constitute research as defined by the act and whether attempts to improve the success of these procedures are thus in violation of the act. Research and experimentation are not explicitly defined in such a way that they may be differentiated from the day to day modifications of techniques. Thus it seems that common sense changes may, by default, become experimental procedures. The urgent need for clarification of this issue has been reflected in headlines in the popular press in Australia to the effect that the Victoria legislation may halt in vitro fertilisation in the state.¹⁵

The scientist's quandary may be illustrated by two simple examples: the first concerns the number of sperm used for fertilisation and the second the choice of culture medium. The usual number of sperm used in in vitro fertilisation is about 50 000.¹⁶ If, however, the sperm seem weak more are taken to ensure that fertilisation occurs. Increasing the number of sperm has several possible consequences; it may increase the occurrence of dispermy or polyspermy,¹⁷ and it may decrease the chances of the zygote surviving in the fertilisation medium (P Rogers, personal communication). Does this mean that varying the number of sperm used can be construed as an "experimental procedure . . . that would cause damage to the embryo . . ."?²

The second example concerns culture of the embryo after fertilisation. About three quarters of eggs fertilised in vitro produce apparently normal embryos, but pregnancy occurs after transfer in only 10-20%.¹⁸ This comparatively low figure implies that modification of the culture medium may be necessary to increase the success rate after implantation. Preliminary trials and animal studies have indicated that fertilisation and subsequent culture in human amniotic fluid, rather than synthetic media, not only decreases the incidence of abnormal fertilisation but also leads to higher rates of pregnancy after transfer.¹⁹ This modification has been successful experimentally, and a large scale clinical trial is in progress. The procedure may be properly tested only in humans, and for final assessment embryo transfer is required. Because transfer is a necessary part of the protocol and indications are that rates of both fertilisation and pregnancy are improved, this particular instance does not constitute an experimental procedure under the act, but how should similar modifications that are unsuccessful be classified? Do they become experimental procedures that "would reduce the prospects of pregnancy resulting from . . . implantation"?²³

Clarification of the definitions of these practices under the act is essential. A line must be drawn between research and practice, defining each but bearing in mind that current techniques do not constitute foolproof recipes; they are still largely experimental themselves. Until such clarification is forthcoming those working in clinical in vitro fertilisation programmes in Victoria are reluctant to continue to practise. A long term consequence of delaying such clarification could be a decline in the efficiency and success of the present programmes, culminating in the loss of scientific skill from the state.

Future prospects

The future of in vitro fertilisation is closely linked with the future of experimentation on embryos, which in turn, in Victoria, depends on interpretation of the current legislation. The former dependence is not new; current clinical in vitro fertilisation programmes developed from animal studies followed by trials using human gametes and embryos in which abnormalities and technical problems with procedures were studied.²⁰ Techniques of transferring embryos were developed in a similar way. Animal models remain the initial step in developing new techniques for repro-

ductive medicine, but as the understanding of early human development increases so we become aware of the differences in development between humans and animals²¹; this indicates a need for experimentation on the human embryo. When such experimentation is not possible new techniques are unlikely to be developed. This in turn raises the problem of whether a partially tested procedure that has been perfected in animals but untried in humans should be offered to patients.

In making the early human embryo accessible in vitro fertilisation has provided the means for further study of the processes of early pregnancy and pregnancy loss, development, differentiation, and fertilisation in humans. It is now possible to address questions whose answers lie outside the scope of animal experiments. In Victoria, however, decisions about whether such questions will be investigated in future are now the responsibility of the Standing Review and Advisory Committee, not the scientists.

Regulating by law the development and direction of in vitro fertilisation programmes and at the same time preserving clinical practice is a difficult task, for as each decision is made it must be appreciated that just as in vitro fertilisation techniques have been instrumental in providing opportunities for further research so research remains an integral part of current clinical practice. The Victoria act has created problems for scientists and for lawyers, and there are many valuable lessons to be learnt from this act by other legislatures: among the most important is that terminology should be clearly defined. If this cannot be done the question arises of whether any such legislation now is premature.

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