In vitro fertilization – a fast changing technique: a discussion paper[1]

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Edwards and Steptoe received acclaim from the general public and the scientific world alike when Louise Brown was born in the summer of 1978. This wonderful achievement enabled a patient to have a healthy child where only a few decades previously this would have appeared impossible (Steptoe & Edwards 1978). The public response stemmed in part from the assumption that man had wrested from nature the control of conception and maybe even of fetal development. In this respect the term 'test-tube baby' has fostered many misunderstandings. Scientifically, however, this event was the culmination of years of endeavour with a technique previously proven in some animal species. Its immediate clinical relevance was the broadening of treatment options for infertility, offering hope to those with irreparable tubal disease and the prospect that pregnancy might be achieved in some other infertile conditions, e.g. cervical hostility, endometriosis, oligospermia and unexplained infertility. It is now possible that other conditions may be treatable with the availability of ovum donation, e.g. inheritance disorders, ovarian accessibility.

In Melbourne Professor Carl Wood's team have been actively researching in this field for many years and their scientific contributions have been of particular assistance to others (Lopata *et al.* 1978, Lopata *et al.* 1980, Trounson, Wood *et al.* 1981, Trounson, Leeton *et al.* 1981, Wood *et al.* 1981). Analysis of independent research, particularly from Australia and the UK, now allows us to gain some insight into what is and is not essential for success.

In January 1979 at a meeting held at the Royal College of Obstetricians and Gynaecologists, Edwards & Steptoe outlined the management that resulted in four out of thirty-two women becoming pregnant following embryo transfer (two did not proceed to term). The methods used differed in some respects from a previous series, and they outlined the measures required to succeed. One was the inclusion of an embryologist in a team. This would be an optimum if funds allowed, but this stipulation is financially impossible if the method is eventually to be available at district hospital level, as it ultimately should and will be. In any event, it is unnecessary, as indicated by the recent pregnancies occurring in this Department, where the laboratory aspects fall well within the capability of a carefully trained technician. Another major health service cost consideration is the nature of ovulation and the ovum capture system. Edwards & Steptoe (1979) previously suggested that their success was related to abandoning the use of drugs to induce ovulation, i.e. HMG, relying on the laparoscopic collection of mature pre-ovulatory ova from natural cycles. We and Professor Wood's team have always held the opinion that ovum collection has to occur at a time convenient to the gynaecologist and laboratory, minimizing the cost of personnel on-call and also patient disturbance (Craft 1980, Lopata *et al.* 1978, Trounson, Wood *et al.* 1981, Trounson, Leeton *et al.* 1981, Wood *et al.* 1981). We have both used clomiphene to promote follicle growth and HCG to time ovulation, aspirating ova 36 hours after the latter. It is pertinent that Edwards & Steptoe (1982) have lately revised their previous dogmatic view and are also using clomiphene/HCG. Recently, Howard and Georgeanna Jones (1982, personal communication) have reported a live birth in the USA using the HMG/HCG combination, and there are other continuing pregnancies.

These interesting findings are quite the reverse of those indicated by Steptoe and Edwards who suggested that HMG therapy used in normally-menstruating women had been

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responsible for their previous lack of success, even though their very first pregnancy – an ectopic – occurred after its use (Steptoe & Edwards 1976). They postulated that abnormal sex steroid production and a shortened luteal phase were responsible (Edwards & Steptoe 1979, Edwards *et al.* 1980). However, in my opinion, endocrinologists and gynaecologists may have put too much emphasis on the association between the shortened luteal phase and decreased fertility. Implicit in this concept is an impression that the follicular phase is good but the luteal phase is bad. I would propose that the opposite concept may be nearer the truth, i.e. in a situation where ovarian follicle development is not optimal poor proliferative endometrial preparation is likely and with it an imperfect secretory response. This will minimize the success of embryo implantation in either an *in vitro* or *in vivo* situation.

Pregnancy is most likely to occur when there is good ovarian follicle development with follicles containing 5–10 ml of fluid and where cervical mucus scores are high. Whether low-dose oestrogen therapy given in the latter part of the follicular phase can enhance the proliferative endometrial response prior to ovum capture so as to maximize the secretory effect of progesterone, and with it increase its receptivity to embryo implantation, is an interesting thought. One can imagine in nature that there are populations of patients who inherently, or at specific times, are good or bad procreators. It will be an asset if we can influence the fertility of the latter by such measures. Certainly, there are no reports that the addition of antiprostaglandins, beta-stimulant agents, HCG or progestogens has any definitive value with *in vitro* fertilization (IVF) when administered in the luteal phase (Wood *et al.* 1981).

There are advantages of using ovarian induction agents other than mere convenience. Their use increases the chance of collecting more than one mature ovum, maximizing the prospect of pregnancy from one operative procedure (*Lancet* 1981). Trounson (Trounson, Leeton *et al.* 1981) has advised inserting up to two embryos and the first twins have now been born in Australia; and we have inserted up to three in the hope of achieving at least one infant. The transferred normal embryo may influence its own ability to implant, but we have yet to establish whether the incidence of twin pregnancy after insertion of two embryos is greater than that of a single pregnancy after inserting one. In our present ongoing twin pregnancy three embryos were inserted, all implanted as detected by ultrasound, but only two fetuses survive, so there may be something facilitatory in inserting more than one embryo.

There may also be some advantage in using HMG rather than clomiphene, although its supervision is generally more complex. Increased follicle stimulation may occur with collection of more ova and if more than 2–3 embryos result, then once cryopreservation is perfected, transfer in a subsequent cycle may maximize the prospect of pregnancy from one operation if the first transfer failed. Cryopreservation may also help when embryos need to be inserted in recipients at the correct stage in the cycle, as would be necessary for women having ovum donation for reasons previously mentioned. HMG therapy may maximize successful ovum capture in some patients with limited ovarian accessibility where only part of the ovary is available for puncture. Even laparoscopic blind puncture of the ovary may be more successful if a follicle is on the unseen distal aspect of an adherent ovary. Percutaneous transabdominal puncture of a follicle under local anaesthesia using ultrasound control has been proposed as an alternative to laparoscopic collection (Lauritsen, personal communication). However, I doubt that this method will be as effective for ovum capture as the laparoscopic procedure for patients with free ovarian accessibility. It would be particularly helpful for those in whom the ovaries are hidden from view.

If, as is my opinion, ovulatory drugs are not responsible for the failure of the technique, what is responsible for the recent successes? A number of factors may be contributory as has been suggested by Trounson. His advice that a collected ovem should be allowed to mature *in vitro* for 4–6 hours before sperm are added is a major consideration (Trounson, Leeton *et al.* 1981, Wood, *et al.* 1981). This results in an increased incidence of fertilization and normal cleavage. One wonders why it was assumed that fertilization would be optimal if sperm were added immediately following ovum collection 36 hours after HCG, whereas *in vivo* this occurs after 40 hours or later. The better preparation of semen by washing, centrifuging and

resuspending in appropriate culture medium several times favours motile sperm. We have found that 10^4 sperm will effectively achieve fertilization where 10^5–10^6 were previously advised (Craft, McLeod, Bernard *et al.* 1981). Even fewer will almost certainly be as effective. This interesting finding has a practical relevance since we have successfully fertilized ova where the semen is oligospermic. Whether IVF is the correct treatment in this situation is debatable, since manipulation and preparation of the semen in an appropriate culture medium for intrauterine transfer of sperm near to timed ovulation may be all that is necessary. Only comparative studies will answer this question, but now some hope exists for the infertile male with oligospermia. Improved laparoscopic collection and embryo transfer equipment has made the system easier and Trounson has advised using Teflon as a catheter material both lining the ovarian aspirating needle as well as for the transcervical embryo insertion catheter. Certainly this aspect has not been stressed sufficiently and some available catheters destroy embryos (Craft *et al.* 1982). Experience in an *in vitro* animal system, e.g. mice, allows familiarity with IVF and a cross-check on the media and equipment used for both animal and human work. A number of well documented media will support embryo growth provided the correct constitution, osmolality, pH, etc. are maintained. Whereas it has been suggested the ovum should be left with the sperm for 12–15 hours and then be subcultured, it is our experience that this latter step is unnecessary. Culture can be effected in dishes or tubes, and with the latter covering the medium by paraffin is unnecessary. Where an embryo has coronal cells condensed around itself, their dissection off the embryo, formerly considered essential prior to transfer, is now considered superfluous.

The optimal stage of embryo development at transfer is uncertain. Edwards & Steptoe (1979) advised the 8–16 cell stage, but we and Trounson have considered that earlier transfer might minimize possible impaired *in vitro* development since it has been shown in monkeys that 2-cell embryos transferred into the uterus will subsequently implant (Marston, personal communication). Our recent studies indicate that even a transferred single cell will develop and implant, which implies the time of incubation may be reduced. However, we cannot be definite as to the optimal time of incubation and of transfer, which will only be determined by assessing the number of successful pregnancies following the transfer of 1, 2, 4, or 8 cell embryos. Occlusion of the tubes at the uterine cornu has been advised by Steptoe since ectopic pregnancies have occurred with IVF (Steptoe & Edwards 1976, Tucker *et al.* 1981), but this has now become the subject of controversy and we feel it should be unnecessary if, as is possible, only 10–15 µl of fluid is injected and the catheter tip is adjusted so it is short of the fundus (Craft, McLeod & Edmonds 1981). It is emotionally stressful for an infertile woman to be sterilized. In any event it would not prevent an interstitial pregnancy. Now Wood *et al.* (1981) have shown that IVF is of value in the management of unexplained infertility, it is even more imperative that sterilization is not undertaken since one failed attempt may be followed by a successful natural pregnancy in a subsequently untreated cycle. As to the time of embryo transfer, Edwards & Steptoe (1979) advised transfer in the late evening but others consider successful pregnancies will result unrelated to the time of day. We do not yet know if there is an optimum time. Similarly, following transfer some advise bedrest for 24 hours and Edwards & Steptoe (1982) have suggested inpatient stay for four days. Neither is likely to be essential for successful implantation and pregnancy. Day care and outpatient attendance may become a reality with laparoscopic or percutaneous ovum collection and embryo transfer respectively.

There is still considerable scepticism that this technique is either safe or is sufficiently efficient to have an accepted place in infertility management. Despite extensive animal studies there is no evidence of a higher incidence of abnormalities in liveborn young, and I predict the same will be true for human pregnancies conceived *in vitro*. Critics point out that in nature only 25% establish continuing pregnancies per cycle assuming all factors are known to be normal, and that this figure should be accepted as the ceiling for IVF since nature knows best. However, the latter is almost certainly untrue, despite the modern preoccupation with this general philosophy. Whilst we do not know what percentage of embryos entering the uterus normally implant *in vivo*, 59% of normal women have positive beta subunit HCG values per cycle (Edmonds *et al.* 1982). The difference between these two figures reflects patients who

'abort' at or around menstruation or subsequently. Similarly, it is unknown what proportion are lost because the embryo is abnormal or the endometrial environment inadequate. Even our acceptance of the figure of 25 out of 100 exposed normal cycles resulting in a continuing pregnancy is probably incorrect since the ovum is not always liberated from a follicle at ovulation, but is occasionally retained (Craft *et al.* 1980), and ovum pick-up by normal fimbria cannot be 100% efficient. In the *in vitro* system some patients also have positive beta subunit HCG values which do not persist, but we may in the future be increasingly able to influence some of the variable factors associated with success, i.e. good follicle development with ovulation agents, correct timing of ovum capture, adequate ovum maturation in culture medium, maximal sperm preparation, nontoxic and traumatic transfer, endometrial receptivity. Even now Trounson (1982, personal communication) has a continuing pregnancy rate per laparoscopy in excess of 20% and the technique can be repeated. A still higher rate may be achieved and these results indicate that IVF must play a significant role in infertility management in the future. Of course, for some forms of infertility its use is not relevant, e.g. anovulation responding to ovulatory stimulants, hyperprolactinaemia treated by bromocriptine, but it is likely to become the treatment of choice for unexplained infertility, cervical hostility and possibly for endometriosis. It may even supplant most surgical remedies for tubal disease. Even now most gynaecologists operate by naked eye or with limited loupe magnification but overall success rates are low. Few are accomplished with microsurgical skills, and even though microsurgery is used for certain types of tube defect IVF may yet become more appropriate in some circumstances. In the absence of tubes, e.g. following bilateral ectopic pregnancies, it is the patient's only hope.

Infertility is an extremely distressing problem for a large number of people and one can understand the patients' concern that they are receiving the most appropriate and effective treatment. IVF should be seen in this context, since once perfected it may be cheaper and more efficient than present practices. However, we cannot suddenly expect a profusion of regional or even district hospitals to provide a service. There are few AID facilities so what hope is there for IVF? In some centres consultants may have too many commitments to subspecialize, and yet technically ovum capture is an easily learned skill. The whole concept of providing this service concerns having a new and wider philosophical attitude to the management of infertility. It should be possible for all teaching hospitals and some district general hospitals to structure their treatment around an infertility clinic which provides various treatment options, functioning every working day of the week. This could be administered by the employment of a nurse working either on a half-day or preferably a full-time basis. It should then be possible to organize patient referrals, postcoital tests, semen analysis, AID/AIH, serum hormone values and even IVF. It is our experience that such a nurse acts as an excellent liaison between the patient and the medical members of the team. The latter should include junior medical staff and at least one well-trained technician. The accurate prediction of incipient ovulation is perhaps the most essential feature of such a programme. Serial plasma or urinary LH and oestrogen assays are time-consuming and expensive but are the most accurate way of determining this end-point. Most hospitals now have ultrasound facilities and it is possible to fertilize mature ova by performing ovum collection 36 hours after HCG given when the follicle is 1.9–2.0 cm in diameter, without monitoring LH or oestrogen values. The success of doing so may be lower than using a combination of both methods. Future ultrasound technology may minimize the need for biochemical assays, but both methods will assist the detection of early pregnancies following embryo transfer.

It is my opinion that the presently held concepts of infertility management will radically change in the near future, and in this respect free dissemination of relevant scientific data and expertise is essential if IVF is to be more freely available. However, it is equally depressing at this time that some members of our profession should, by their recent public statements, be seen to minimize the chance of a couple obtaining that which they prize most – a child. The recent comments of a representative of the BMA Ethical Committee made on television and reported in the Press are a good case in point. All those working with IVF are concerned about ethical issues since their prime objective is to help their infertile patients become

pregnant; and they are anxious to protect their patients and indeed themselves from unfair or ill-informed criticism. Alarmist comments made before fully establishing contacts with all those working in this field are unnecessary. Inadequate knowledge has led to one spokesman being quoted as saying that researchers have the ability to maintain life in a test-tube for weeks or even months! These comments are quite fictitious and contrary to our intention of replacing embryos in the uterus at a very early cleavage stage. Similarily, cryopreservation, limited by time, is a means of maximizing the chance of pregnancy for a particular couple from a single operation, and yet antagonists have promoted it as a means of maintaining life for centuries – for what purpose? Donated ova may be the only way of helping some women to have children, and in September 1979 I expressed the opinion that the Royal College of Obstetricians and Gynaecologists should consider setting guidelines in the same way as it had for AID (Craft & Yovich 1979). It is an irony that the Department of Health, Education and Welfare in the USA should have considered IVF in great detail with doctors, lawyers, the lay public and theologians in 1978 when sanctioning this method of treatment and its related research three years before the birth of the first infant in that country, whereas in the UK no comparable forum has occurred three years after the world's first birth.

I would strongly suggest that those concerned with ethical matters should consider the aspirations of infertile patients. As with many other issues it will be the public who will insist on IVF being made available since it would be unethical to withhold its use. Those promoting a moratorium will have difficulty in persuading the patients who have no other prospect of having a child that they should not be offered IVF.

Research on early human embryos is a more emotive subject. *In vitro* culture can only be sustained for a few days and without a recipient uterus continued development cannot occur. The latter consideration is also true for the vast population of IUCD acceptors where we acquiesce to the fact that embryos are repeatedly discarded each month, being denied the chance of implantation. Whilst my Department is not actively involved in research on early human embryos, it is not illogical that it should be undertaken, provided that the intended studies satisfy the ethical committee standards of the hospital concerned and of the relevant professional medical bodies, and provided that the patient has given her consent. It would be an irony if our profession or its advisers were to limit our knowledge about early human development, which could be of great importance in identifying causes of abnormalities or diseases that we spend so much time trying to treat.

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