SPECIAL ARTICLE

IVF and New Technology

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Historical beginnings

Human IVF began in the late 1960s when Robert Edwards in Cambridge, England, first fertilized human eggs with human sperm in the laboratory[1]. Dr Edwards had made contact with a gynaecologist, Dr Patrick Steptoe, who had pioneered the use of laparoscopy in Britain, recognising that Steptoe's procedures allowed human eggs to be obtained from the ovaries. After that there were eight or nine years of frustration before the first pregnancy was achieved in 1976: the frustration was worse when that first pregnancy turned out to be an ectopic pregnancy[2]. The first baby, named Louise Brown, was not born from IVF until 1978[3], nine years after human IVF embryos had first been described, and this shows just how difficult the technology was to develop. For IVF to be successful every part of the clinical and laboratory procedure must be exactly correct; but on the other hand if everything *is* correct then we now know that it *will* work, and it will work immediately, and it will work predictably.

Australia was introduced to the milestones of IVF history in 1980. The fifth and sixth successful IVF pregnancies in the world were in Melbourne[4], and for the next year or two most of the world's IVF babies were Australian, as Professor Carl Wood and Dr Alan Trounson introduced controlled ovarian stimulation and timed follicle aspiration to clinical IVF procedures[5]. In 1983 Australia had the first pregnancy from transfer of an embryo that had been frozen and then thawed[6].

In 1984 a new technique was introduced which, for the first time in IVF technology, made use of the fallopian tube in women whose tubes are normal. The technique was developed in San Antonio by a colleague, Dr Ricardo Asch, with whom I had worked on the fallopian tube in 1980.

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Instead of leaving egg and sperm in the laboratory he transferred the eggs and prepared sperm into the fallopian tube at the same laparoscopic or mini-laparotomy procedure that had been used to obtain the eggs[7]. Immediately there were pregnancies. His daughter helped him name the revolutionary technique "gamete intrafallopian transfer", or GIFT. Around the world today IVF programs, good and not-so-good ones, consistently show better pregnancy rates with GIFT than with IVF and uterine embryo transfer.

However, in the same year, 1984, a major change was taking place in IVF. Pierre Dellenbach, working in France, showed that follicles could be aspirated and eggs obtained through the mucosa of the vaginal vault, guided by ultrasound[8]. Even more importantly, Dellenbach showed that general anaesthesia was not required for egg retrieval. Around the world, by 1986, numerous ambulatory care IVF programs had been established, requiring no hospital admission. Our own first program, at Royal Prince Alfred Hospital, began in February 1986 and there were pregnancies in the first month of operation. At the same time we also began laparoscopic GIFT at Sydney IVF, also with pregnancies straight away[9]. The laboratory technology that made these immediate results possible was that of the very well established IVF program at the Royal Women's Hospital in Melbourne, and shows the importance of correct introduction of new technology.

In 1987 a variation of GIFT was introduced. Known by such names as "zygote intrafallopian transfer" (ZIFT)[10], "pronuclear-stage transfer" (PROST)[11], "tubal embryo-stage transfer" (TEST)[11] and "laparoscopic fallopian embryo transfer" (LAPFET), these procedures meant that, instead of unfertilized eggs and sperm, fertilized eggs or pre-embryos were successfully transferred to the tube. This technique was - and is still - especially valuable for the treatment of couples where there is oligospermia: it combines the advantages of GIFT (development of the early embryo in the tube and correct transport into the uterine cavity) with the advantages of IVF (confirmation of fertilization before transfer). In 1987 we introduced, as the Chairman kindly pointed out, the technique of catheterizing the fallopian tube from the vagina[12], making "ultrasound" or "transcervical" GIFT possible.

In the most recent breakthrough, in 1988 in Singapore, the world's first pregnancy occurred from sperm microinjection for severe male infertility[13]. In this technique individual sperm cells are placed in the perivitelline space between the zona pellucida and the egg cell membrane. We reported normal karyotypes among embryos produced this way[14] and went on to produce with we think would have been the world's second and third babies from sperm microinjection[15]. I will elaborate on sperm microinjection below.

This, then, is an introduction to

the time-frame in which technology has been introduced to IVF so far. It gives a preliminary idea of how new technology has evolved and how existing technology can be put to new use.

Physiological considerations

In normal reproduction, sperm move from the vagina through the cervix, through the uterus and fertilized the egg in the fallopian tube[16]. The egg has been ovulated from the ovary and will reach the ampullary isthmic junction in about 30 minutes. The egg will be fertilizable for about twelve hours. The sperm in the tube are very few, may be not more than five or ten-a contrast to the millions ejaculated at the start, and the hundreds of thousands that reach the uterus. Why are so few sperm needed in the tube naturally when thousands must be placed in the tube for GIFT to work ? If only we knew !

The fertilized egg stays at the ampullary-isthmic junction for another two or three days before travelling down the tube to the uterus. While still in the tube the fertilized egg develops to the stage of a morula, a solid ball of cells. As it enters the uterus it will become a blastocyst.

With in-vitro fertilization we cannot yet achieve this. Embryos will not divide as quickly as this with present culture technology. Instead with IVF we know from experience that if culture technology in an IVF program is good then we have the best results if we transfer in two days or three days: usually the embryo is only at the stage of two cells or four cells at the time of the transfer. When laboratory conditions and technically not perfect then better results come from transferring on day 1, at the pronuclear stage, than on day 2 or 3[17], although the pregnancy rates will be significantly lower than day 2 or 3 transfers in better laboratories. For good IVF laboratories too-but especially in not-so-good IVF laboratories-the difficulty is that the embryo does not grow as fast in the laboratory as it does in nature, and the longer we keep the embryo in the laboratory the more behind the embryo gets.

While IVF embryos lag behind in development, this is not the case for the uterus. The sequential action of progesterone on the endometrium is well known to all gynaecologists. If you look at the endometrium on day 16 or 17, when we should be transferring the embryo, the endometrium will normally be developing the first histological signs of secretion, namely basal vacuolation. But in stimulated IVF cycles we have found on endometrial biopsy at the time of embryo transfer that the endometrium is advanced: the stroma may look like day 21, the endometrial glands may look about day 18 or 19 (Jansen RPS, Anderson JC, and Russell P, unpublished information). The endometrium is both abnormal and advanced. So with IVF on the one hand we have the endometrium which is developing too quickly, there is too much progesterone effect. On the

other hand we have the embryo which is slow . Embryo transfer studies in other mammals, in which synchronous and asynchronous transfers are compared, show that the embryo can wait, if the endometrium is behind, but if it is the embryo that is behind and the endometrium is ahead then the embryo often cannot catch up. This is the single most important limitation on IVF today. For IVF to work acceptable we need to stimulate the ovary to bring more that one follicle to yield mature eggs, but when we stimulate the ovaries we get an endometrium which behaves as if there is too much progesterone, it develops too far too fast, and the embryos cannot often catch up, which is probably why only a small proportion of IVF embryos result in viable fetuses, even with the best of present technology.

Present culture techniques will not bring embryos to the blastocyst stage on time. Attempts have been made to improve embryo development in vitro by trying to duplicate what the tube can do with epithelial co-cultures[18], but improvement is so far only marginal.

A second difficulty with IVF is the mechanical difficulty, sometimes, of transferring embryos through the cervix and its mucus. We have found that it does help to use ultrasound to see the catheter in the cavity of the uterus for accurate transfers, but still there may be translocation of the embryos away from the deposition site as the catheter is withdrawn.

The advantages of utilising the tube for GIFT, ZIFT, PROST and so on are therefore several. First, laparoscopic deposition of gametes or embryos through the fimbrial end of the tube to the AIJ is mechanically more reliable than transfers to the uterus. Second, early embryo development in the tube is likely to be normal instead of slow (although the endometrium is still relatively advanced). Third, entry of embryos into the uterus from the tubes is more correct than introduction through the cervix.

The trouble since 1985 has been, however, that, just as laparoscopic tubal transfers were being shown to have important advantages over uterine embryo transfers, IVF itself had moved away from laparoscopy to a walk-in, walk-out outpatient basis utilizing transvaginal ultrasound, with the patient remaining awake for the procedure. So Dr John Anderson and I developed a catheter system in order to transfer eggs or embryos into the fallopian tube by ultrasound.

Ultrasound-guided transvaginal catheterization of the tube

The KJITS-2000 catheter system (Figure 1) consists of (a) an outer opaque Teflon canula, which is placed by ultrasound control (or slightly less reliably by tactile sensation alone) [REF] at the lateral angle of the endometrial cavity, and (b) an inner clear Teflon tubal catheter, of external diameter 0.6 mm. We reported the catheterization technique in 1987[12],

pregnancies from artificial insemination using the catheter in 1988[19], and the world's first pregnancy from embryo transfer through the catheter in 1988[20]. Our general experience has been that the chance of pregnacy with ultrasound-GIFT or ultrasound-fallopian embryo transfer (ultrasound-FET) is, with current techniques, about two-thirds that which we get with laparoscopic cannulation of the tubes from the fimbrial end.

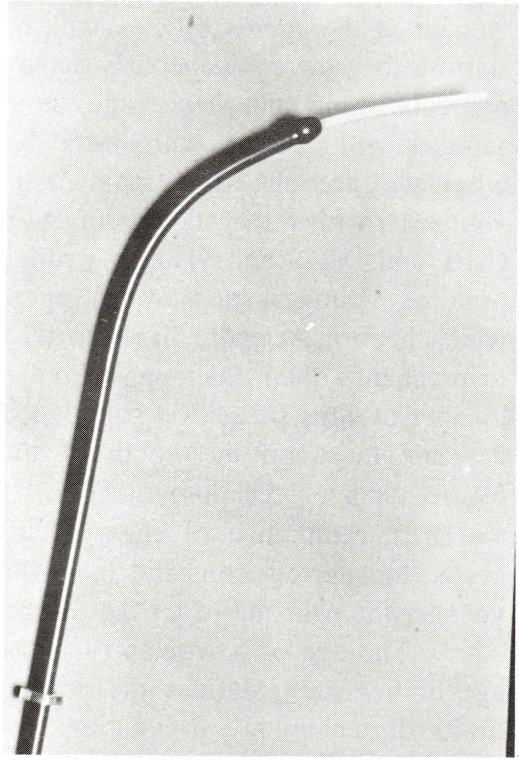

Fig. 1 KJITS-2000 fallopian tube catheter set (William Cook Australia Pty Ltd., Brisbane Technology Park, Eight Mile Plains, Queensland 4113, Australia) consisting of an outer bulb-tipped cannula, which reaches the lateral angle of the endometrial cavity, and an inner fine catheter, which passes through the uterotubal junction into the fallopian tube.

Ovarian stimulation

The more embryos that are transferred the higher the pregnancy rate. That means that for IVF and GIFT procedures to have a good chance of success in practice we should stimulate the ovaries. In stimulating the ovaries with human menopausal gonadotropin (hMG) injections we do what we can to prevent the LH surge. In principle, we can use hMG alone, hMG with clomiphene, or hMG with GnRH analogs: with all but the last of these regimens spontaneous LH surges compete with exogenous human chorionic gonadotropin (hCG) injections to initiate ovulation.

Clomiphene increases pituitary FSH release and has several advantages. One is that it is cheap. Second, because it is an anti-estrogen, it holds the endometrium back a bit and that is probably good for IVF. The disadvantages are (a) that it does not prevent the endogenous LH surge, so even with careful monitoring some stimulation cycles will be spoiled by unscheduled ovulation, and (b) that the retarded endometrium is qualitatively abnormal, increasing the chance of faulty implantation and spontaneous abortion.

GnRH analogs such as leuprorelin (or leuprolide in the United States) inhibit pituitary gonadotropin secretion and thus prevent both endogenous LH surges and clomiphene-induced abnormalities in the endometrium. When we introduced leuprorelin at Sydney IVF we notice an overall

improvement in our GIFT pregnancy rates[9], even though we reduced the number of eggs from four down to three (Figure 2). We also noticed that the chance of miscarriage was less than on clomiphene.

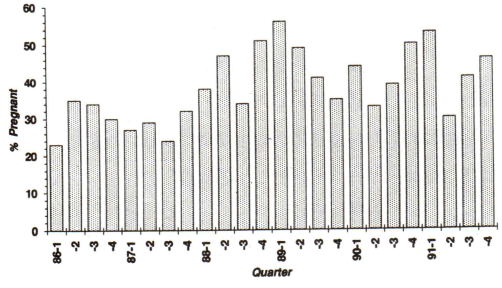

Fig. 2 Pregnancy rates by quarter for laparoscopic GIFT at Sydney IVF since start up in 1986. The introduction of leuprorelin instead of clomiphene in association with hMG in the fourth quarter of 1988 maintained the pregnancy rate despite a reduction from 4 to 3 in the number of eggs transferred.

Female limits to IVF: Egg or embryo storage

The more eggs we get the better the chance of pregnancy. But even if we transfer only three or four eggs we still see that the more eggs we get the better the chance of pregnancy. So it is not just the number of eggs we transfer but the more eggs we get the better the chance of pregnancy - even though we just transfer a maximum of three eggs. Why is this ? The reason is that a woman, when she is a 20 weeks fetus, has up to about eight million eggs, all contained in primordial follicles, after which there is a steady decline in number of eggs in the ovaries; she produces no new eggs; the eggs that are there get older and older; there is always a proportion of the follicles starting to develop, but until puberty and the commencement of ovulatory cycles all of these follicles undergo atresia and the eggs are lost. By the time she is born she has only one and a half million eggs. By the time she is a teenager she has about 300000 eggs. The younger a woman is the more follicles will be starting to grow spontaneously at any particular time and hence the more follicles will develop with hMG. So what we are seeing is that it is younger ovaries that do better with GIFT and IVF - and younger ovaries produce younger (and more) eggs, which in turn are more likely to lead to pregnancy than older eggs. So the number of eggs we get on stimulation depends more on the youth of the ovaries than on the dose of hMG. If we keep the number of eggs or embryos transferred constant then the younger the eggs the better the results.

The age of a woman (and the age of her eggs) defines the present limits of technology in treating women: the clock cannot be turned back ! How do we help in the future ?

The answer will probably be to obtain eggs at a young age and store them. With existing technology this is possible only with ovarian stimulation, egg recovery, IVF and then embryo cryostorage. But what about unmarried women ? This is almost science fic-

tion now but it is likely that in the future those wealthy women who can afford it will be able to choose to keep some immature eggs frozen from the age of 25 for 10 or 15 years, until they want to get pregnant. The eggs may need to be at the germinal vesicle stage to resist freezing and thawing without damage.

Male limits to IVF: Sperm microinjection

The limitation of IVF for men is overcoming the very low sperm count. How can new technology in IVF help the man with the low sperm count ?

Figure 3 shows fertilization with GIFT, standard IVF and IVF by sperm microinjection at Sydney IVF. With GIFT we do not get pregnancy with fewer than about 50000 motile sperm per drop (30 ul); with IVF, we do not get fertilization with fewer than about 8000 motile sperm pre drop; but with sperm microinjection into the perivitelline space (Figure 4) fertillization is possible with much lower sperm counts after sperm preparation.

As mentioned above, the first pregnancy from sperm microinjection took place in Singapore[13]. Sydney IVF's first two pregnancies, in 1990, were among the next to occur[15]. We now have more than 10 successful pregnancies and the pregnancy rate in 1991 was 11.9% of initiated cycles, all among couples with desparately low sperm counts and previous unsuccessful conventional IVF. But the fertilization rate with microinjection is still relatively low at about 15 to 25% of eggs fertilized. We need to ask why this is. Where are the technological difficulties at the moment with sperm microinjection ?

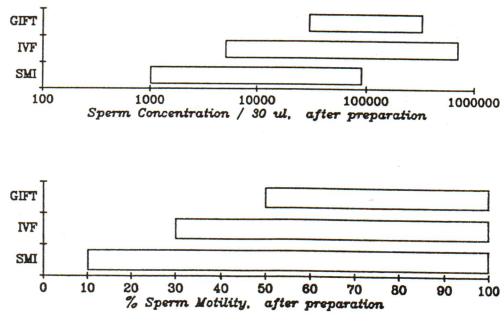

Fig. 3 Sperm concentrations per 30 ul and motility of prepared semen samples associated with fertilization at GIFT, conventional IVF and IVF by sperm microinjection at Sydney IVF.

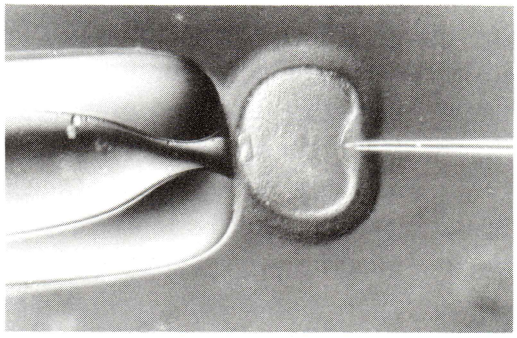

Fig. 4 Sperm microinjection below the zona pellucida into the perivitellin space. Photographed by Janine Lippi, Sydney IVF.

Firstly, we were worried that by collecting just one sperm and putting it next to the egg we were forgetting Nature's role in sperm selection. So ethically, in introducing this new

technology, we had to do experiments on the embryos before we could begin clinical procedures. This required approval from the Ethics Review Committee of the Central Sydney Area Health Board-the first time in Australia that and institutional ethics committee had given explicit approval for embryos to be created *solely* for the purpose of research. We compared the results of sperm microinjection with karyotypes of embryos on conventional IVF and we found that the abnormality rate was only 22% with fertilized injection and 30% for ordinary IVF[14]. So we and our institutional ethics committee were happy with that and we proceeded then with the clinical studies.

Secondly, sperm during transit of the male and then the female reproductive tract are protected by an acrosome. Once capacitated, sperm are capable of undergoing the acrosome reaction - an event the normally occurs upon binding of the sperm to the zona pellucida. Excretion of the acrosome releases enzymes that digest a path through the zona. More importantly, here, is that the loss of the acrosome reveals binding sites on the inner acrosomal membrane that allow the sperm to bind to the egg cell plasma membrane - the first step directly leading to fertilization of the egg. When we do microinjection we inject the sperm through the zona, and the sperm generally will still have the acrosome: they will therefore not bind to the egg. By manipulating culture conditions we can increase moderately the proportion of sperm in a prepared sperm sample that have undergone the acrosome reaction spontaneously[21], but because the proportion of sperm naturally acrosome reacted but still healthy is low the fertilization rate with sperm microinjection is also low.

We do not yet have a solution to this problem of injecting only acrosome-reacted sperm, but Sydney IVF and other research groups are looking at several possibilities, including application of a high voltage electric current on the sperm, called electroporation or electrofusion. This is like applying an electric shock to the sperm to lose the acrosome reaction. We do get some success with increased acrosome reaction but at the same time sperm motility falls very briskly, so that this is one of the technological frontiers in this area of sperm microinjection.

Clinical indications for assisted conception

When should we introduce technology to getting pregnant for individual couples ? Getting pregnant is sometimes easy and sometimes difficult. To decide if IVF or GIFT or an assisted conception procedure is the right decision for infertile couples we need to consider the mathematics - in particular we need to consider the statistic known as *fecundability*, the monthly chance of getting pregnant. Like everything in nature, its value has a distribution across the population. The average is about 20% per

month, or as a proportion, 0.2. But the extremes range from about 3% per month to about 50 or 60% per month. At the low end of this range the chance of pregnancy, even though "normal", may only be 20% per year, so many of these normal couples will see the doctor with infertility. On the other hand there are other people who get pregnant very easily and will still be fertile when there are one or two infertility factors present.

When we investigate infertility we may or may not come up with a diagnosis. If there is azoospermia, or anovulation, or genital tract occlusion, we have a reason for complete infertility - for sterility. Otherwise we have a situation of relative infertility. Choices of treatment for sterility or for infertility then consist in principle of (1) an attempt at cure (e.g. an operation for fallopian tube obstruction), or (2) to resort to assisted conception. IVF was invented to overcome irreparable fallopian tube disease, but from the beginning it also had the advantage that it can overcome many different causes of relative or relative infertility at once - e.g. by overcoming a low sperm count, in overcoming endometriosis, in overcoming peritubal adhesions, in overcoming cervical factors. This means that IVF and assisted conception may be indicated in many more cases than were originally intended. It is especially useful when there are several things wrong at once (Table I). If you have some decrease in sperm count, some endometriosis, some peritubal adhesions, and you do GIFT then the chance of pregnancy is just as good as it is in any one - or none - of them.

So the question of whether or not to use assisted conception to help a couple get pregnant does not depend so much on the exact diagnosis. Instead, it depends on *time:* first, on the amount of time the couple has spent

Table 1 Effect of one or more theoretical infertility factors on the monthly and yearly chance of natural conception, and on the average expected time to achieve concpetion. Assisted conception procedures often have an equal chance of working irrespective of whether such infertility procedures are present or not.

No. infertility factors*	Monthly probability	Yearly probability	Av. time to conception
0	20 %	94 %	4 months
1	4 %	40 %	2 years
2	1 %	10 %	7 years
3	0.2 %	2 %	40 years

* each factor, such as endometriosis, oligospermia, peritubal adhesions, cervical disease, polycystic ovary syndrome etc. is assumed to be of such severity that it decreases the monthly chance of conception to one fifth of what it would otherwise be.

in trying to achieve pregnancy (the duration of the infertility); and second on the amount of time that is left to get pregnant (how old the woman is, and the urgency of getting pregnant). Once a decision is made to use assisted conception the question becomes, What form of assisted conception is best? The answer here is to make use of the tube if it can be shown to be normal (e.g. GIFT) and to use IVF instead of GIFT if (a) there is doubt about sperm function (the embryos can still be transferred to the tube, e.g. ZIFT, PROST, FET), or, of course, (b) if the fallopian tubes are missing or are damaged (when uterine embryo transfer is the only option).

Now, if assisted conception is cost effective in a rich country like America then it is in principle cost effective everywhere. In every country where infertility is important you must use those methods of getting pregnant that are most effective, and if it is cost effective to do IVF in one country where costs are high, it can also be cost effective in another country where many of the costs are much lower. A developing country has even scarcer resources to waste on ineffective treatment that a rich country has. Once the capital equipment for an IVF program has been bought then the item that most determines the cost of providing assisted conception services is the price charged for hMG (for Humegon from Organon and for Pergonal from Serono), which are about the same real price in every country in the world, and so are relatively much more expensive in poorer countries than in rich countries. The costs of producing hMG are set to fall soon as both companies adopt gene technology (recombinat DNA technology) to synthesize FSH and LH instead of having to extract hMG from the urine of postmenopausal woman. More than any other single factor, the availability of accurate and effective assisted conception will depend on the pricing policies these companies adopt for their product in areas of the world that are not yet rich.

Introducing assisted conception technology

Because IVF and related assisted conception procedures are almost always expensive for patients you would think that great care and other peoples' experience would be important when new programs are started. Unfortunately, this is not always so. Because infertile couples are so desparate that they often will pay for assisted conception when those who are providing it have not yet brought themselves up to the high standard that is required in every single aspect of the IVF process before pregnancy becomes likely. The cost of the doctors' and scientists' education is paid for by the patients, who can least afford it. But this slow and cruel development of IVF is becoming harder and harder for develpoing IVF programs to follow, because the patients in poorer countries who can afford IVF can now

usually also afford to travel to a centre where IVF practices are already well developed. It is therefore essential that a city like Bangkok should develop an IVF program that is immediately good enough for rich patients form Bangkok to stop travelling elsewhere for treatment. Unless the patients have the confidence to stay in Bangkok, the doctors and scientists and nurses involved with IVF in Bangkok will not have the confidence and experience to improve IVF facilties for the poorer citizens who cannot readily afford to travel, for example, to Singapore for treatment.

It is important to realise, first, that, outside Riyadh in Saudi Arabia, government funding alone has not been enough anywhere for a successful IVF program to be established - not in Britain, not in Australia, and not in the United States, so it is unlikely that you will ever find public funds sufficient to develop IVF properly in Bangkok. In Cambridge it was a private venture at Bourn Hall by Steptoe and Edwards that popularised IVF (although they built a large private hospital just as IVF became an outpatient procedure, and the venture was apparently sold to the Serono company to enable it to stay in business). In Melbourne, IVF developments at the universities were always supplemented by the fees earned from private IVF practice. Sydney IVF is a private IVF program that works in close association with a public hospital, Royal Prince Alfred Hospital, to make IVF available to people who cannot afford private IVF. Around the world, all the major developments have taken place with the co-operation and the cash flow generated by the private side of IVF, so that patients who are the wealthiest are the first to be treated, but they give the IVF program the momentum to develop. Most of the important research has been done in this joint environment between the private side of IVF and the public hospital side.

After developing ultrasound-based outpatient GIFT and FET procedures and after achieving success with sperm microinjection, Sydney IVF in 1989 began its overseas consultancies. Our experience in the commencement of two IVF programs in Sydney, one IVF program in Jeddah, Saudi Arabia, one IVF program in Kota, India, and numerous short consultancies to troubleshoot IVF programs with problems elsewhere in the world leads to some interesting conclusions on how to introduce IVF technology to an existing infertility treatment program.

In each of the IVF programs we have established, pregnancies were achieved in the first month of operation, so we can safely conclude that techniques that work are fully transportable. We have also developed over the past year or two ways of analyzing why other programs have difficulties, why pregnancies don't happen.

Usually the equipment is perfect. When we travel we see the same equipment everywhere, whether IVF programs have good or bad results. The space in the lab is usually not a

problem: some of the most successful IVF laboratories are very small. Instead it is the way things are done that is usually the problem - the way in which the egg and embryo are looked after is in principle difficult and is in practice often wrong. For the unfertilized egg, protected chemically by the buffering capacity of the cumulus mass, temperature is absolutely critical: 10 minutes at room temperature is enough to destroy the spindles necessary for correct later development[22]. For the cumulus-free embryo, temperature is still important but now the chemical enviroment, including pH and the availability of the correct nutrients and the absence or toxins that can easily diffuse accross the zona are important. Figure 5 shows the micromonitoring equipment Sydney IVF has developed to measure temperature, pH, pCO_2 and relative humidity of the microenvironments into which eggs and embryos are put.

The macro - and micro - environment must be very carefully controlled. Figure 6 shows the temperature in a well-controlled incubator; the incubator is correctly set at 37.5 and manipulations to it makes no difference to the internal temperature where the eggs and embryos are kept; this is a good situation. Figure 7 is an example from a consultancy that we did in the middle east and we recorded the temperature of the heated microscope stage used for looking at the egg and embryos: it fluctuated between 31 and 45 degrees - enough to damage egg spindles from the cold at one extreme and to coagulate egg cytoplasm at the other !

In the future, we can look forward to continuous monitoring of the health of eggs and, especially, embryos, in much the same way as today we monitor newborn babies when they are born.

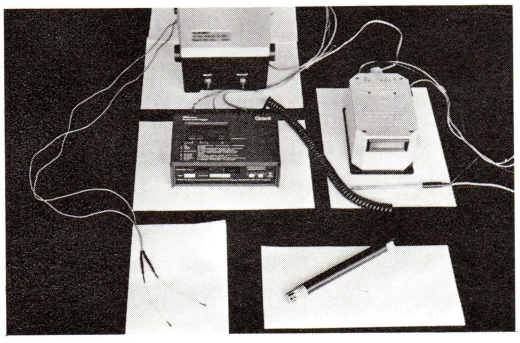

Fig. 5 Sydney IVF microprobes for monitoring temperature (2 channels), pCO_2, pH and relative humidity in the microenvironment of eggs and embryos, and the "squirrel" used for continuous logging of data.

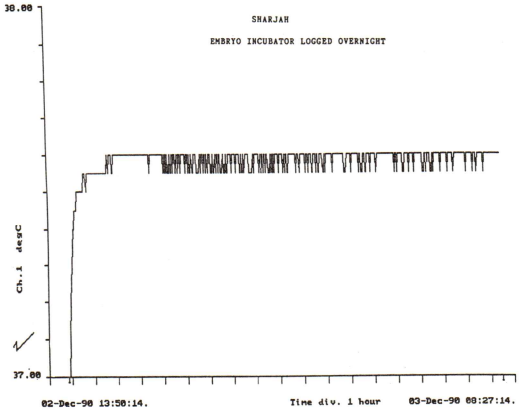

Fig. 6 Temperature monitoring of the embryo section of a water-jacketed incubator. Temperature fluctuations are minimal and acceptable.

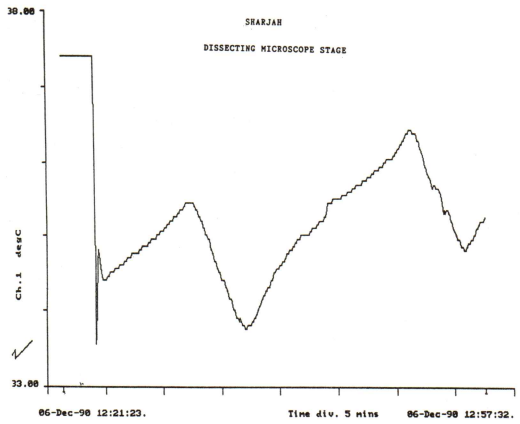

Fig. 7 Temperature monitoring of a heated microscope stage used for identifying oocytes and for preparing embryos for transfer. Damage to oocytes and embryos is inevitable with such fluctuations of temperature.

Cooling of eggs between recovery and entry into the incubator is one of the commonest mistakes we find and it can be very difficult to show where the problem lies (and if it has been corrected) without the solid-state monitoring equipment of the kind developed at Sydney IVF. Without such monitoring the problem may be unsuspected, because the fertilization rates of the eggs affected by cooling may not be reduced; the 2-cell, 4-cell, 8-cell stages may be reached more or less on time; but among the few pregnancies that happen there are many, many miscarriages. The same subtle consequences follow exposure of the early embryo to suboptimal chemical environments.

So clinical IVF programs can believe they are near perfection when they achieve some pregnancies, most of which end in miscarriage. The truth is that very many steps in the process may be suboptimal, but all will require correction before high pregnancy rates follow, and before patients who can pay for IVF stay at home instead of travelling to other countries for their assisted conception.

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References

1. Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilization **in vitro** of human oocytes matured **in vitro**. Nature 1969; 221:632-5.
2. Steptoe PC, Edwards RG. Reimplantation of a human embryo with subsequent tubal pregnancy. Lancet 1976; i:880-2.
3. Edwards RG, Steptoe PC, Pudy JM. Establishing full-term human pregnancies using cleaving embryos grown **in vitro**. Br J Obstet Gynaecol 1980; 87:737-56.
4. Lopata A, Johnston IW, Hoult IJ, Speirs AL. Pregnancy following intrauterine implantation of an embryo obtained by in vitro fertilization of a preovulatory egg. Fertil Steril 1980; 33:117-20.
5. Trounson AO, Leeton JF, Wood EC, Webb J, Wood J. Pregnancies in humans by fertilisation in vitro and embryo transfer in the controlled ovarian cycle.

Science 1981; 212:681-2.
6. Trounson AO, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature 1983; 305:707-9.
7. Asch RH, Ellsworth LR, Balmaceda JP, Wong PC. Pregnancy after translaparoscopic gamete intrafallopian transfer. Lancet 1984; ii:1034.
8. Dellenbach P, Nisand I, Moreau L, et al. Transvaginal, sonographically controlled ovarian follicle puncture for egg retrieval. Lancet 1984; i:1467.
9. Jansen RPS, Anderson JC, Birrell WRS, et al. Outpatient gamete intrafallopian transfer: 710 cases. Med J Aust 1990; 153:182-8.
10. Devroey P, Braeckmans P, Smitz J, et al Pregnancy after translaparoscopic zygote intrafallopian transfer in a patient with sperm antibodies. Lancet 1986; i:1329.
11. Yovich JL, Blackledge DG, Richardson PA, Matson PL, Turner SR, Draper R. Pregnancies following pronuclear stage tubal transfer. Fertil Steril 1987; 48:851-7.
12. Jansen RPS, Anderson JC. Catheterisation of the fallopian tubes from the vagina. Lancet 1987; ii:309-10.
13. Ng S-C, Bongso A, Ratnam SS, et al. Pregnancy after transfer of multiple sperm under the zona. Lancet 1988; ii:790.
14. Kola I, Lacham O, Jansen RPS, Turner M, Trounson A. Chromosomal analysis of human oocytes fertilized by microinjection of spermatozoa into the perivitelline space. Hum Reprod 1990; 5:575-7.
15. Lippi J, Turner M, Jansen RPS. Pregnancies after in vitro fertilization by sperm microinjection into the perivitelline space. Fertil Steril 1990; 54:s29.
16. Jansen RPS. Endocrine response in the fallopian tube. Endocrinol Rev 1984; 5: 525-51.
17. Quinn P, Stone BA, Marrs RP. Suboptimal laboratory conditions can affect pregnancy outcome after embryo transfer on day 1 or 2 after insemination in vitro. Fertil Steril 1990; 53:168-70.
18. Bongso A, Ng S-C, Ratnam SS. Co-cultures: Their relevance to assisted reproduction. Hum Reprod 1990; 5:893-900.
19. Jansen RPS, Anderson JC, Radonic I, Smit J, Sutherland PD. Pregnancies after ultrasound-guided fallopian insemination with cryostored donnor semen. Fertil Steril 1988; 49:920-2.
20. Jansen RPS, Anderson JC, Sutherland PD. Nonoperative embryo transfer to the fallopian tube. N Engl J Med 1988; 319:288-91.
21. Mortimer D, Curtis EF, Camendzind AR, Tanaka S. The spontaneous acrosome reaction of human spermatozoa incubated **in vitro**. Hum Reprod 1989; 4:57-62.
22. Pickering SJ, Cant A, Braude PR, Currie J, Johnson MH. Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte. Fertil Steril 1990; 54:102-8.