

## Human in vitro fertilization and embryo transfer

Abstracts of the lectures held at the meeting in Torino (Italy), 30 April 1983

### Human in vitro fertilization (IVF) and embryo transfer (ET): an Introduction

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In recent years the greatest novelty in therapy of conjugal sterility has been the in vitro fertilization (IVF) of human gametes, followed by embryo transfer (ET) into the uterus. Many events could be mentioned as notable in the history of this procedure. Briefly, in 1944 the Americans Rock and Menkin obtained the first fertilization and cleavage of human oocytes in vitro. In 1959 Chang produced the first successful fertilization of rabbit eggs in vitro followed by normal pregnancy after transfer. The first human pregnancy clinically verified after IVF-ET was obtained by the English workers Steptoe and Edwards in 1976, after several years of attempts. Unfortunately it was an extra-uterine pregnancy. But the efforts by the same group continued and led to the first birth of a child after IVF-ET in 1978, in England. In 1980 the Australian team of Lopata repeated the success of the English group. The birth of other IVF-ET children followed in Australia in 1981 (the work of the group at the Monash University of Melbourne) and in the USA (the work of the group at Norfolk). In 1982 there were other verified births of IVF-ET children in other countries such as France, Germany, Austria and Sweden. A recent estimate shows a total number of about 200 IVF-ET births since 1978. Following the era of international and national premières, the practice of IVF-ET as a therapy of conjugal sterility is now entering the phase where it becomes routine. All the groups, continuing or just starting with IVF-ET, are confronted with an increasing number of demands for this therapy, which requires a considerable organizational effort.

There are many technical factors that can influence the success of this type of therapy. The following abstracts deal with those factors of the greatest relevance: the selection of patients, the management and monitoring of the cycle, the recovery of the oocytes, the technique of the in vitro fertilization itself, the embryo cleavage in vitro, and finally the technique of the embryo transfer.

### Organization of an IVF program

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Since the birth of Louise Brown, in 1978, more than 200 children have been born after in vitro fertilization. The current progress of these techniques proves the necessity of creating an IVF center. The organization of an IVF program includes a clinical part and a laboratory one. Today there are so many requests for consultations that a preliminary selection of patients has to be made on the basis of the data supplied. Women are then pre-registered on monthly schedules. Ovulation monitoring, as we practise it (Citrate of Clomiphene + HMG), requires ultrasonography and daily hormonal determinations, which are carried out during a period of hospital admittance. At the present, the apparent cost of an IVF attempt is FFfr. 14,000 (a tuboplasty costs FFfr. 15,000). However, these figures do not take into account the rate of success. The IVF costs could be reduced with the institution of IVF centers with ovulation monitoring on ambulant patients, and with simplified oo-

cyte collection methods, by laparoscopy under local anesthesia, or collection under ultrasonography. Such an IVF center would have to include a clinical unit, 15 beds and 3 physicians, a laboratory for embryo culture with 4 technicians or biologists, and facilities for carrying out ultrasonography and hormonal determinations. Apart from its functions in research, such an IVF center could treat many sterility problems.

### Patient selection for IVF-ET

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*I. Prerequisites:* General examination and vaginal bacteriological examination; Familial anamnesis for genetic diseases; Psychological evaluation of the couple; Age of women < 40; Absence of current P.I.D.; Absence of obesity; Ovulatory cycles with normal endocrine profiles; Accessibility of ovaries; Normal uterus; Satisfactory seminal fluid (at least 20 times  $10^6$  spermatozoa with progressive mobility per ejaculate, mobility of grade 3-4 in at least 40% after in vitro capacitation, phlogosis absent or cured).

*II. Indications:* Irreparable tubal damage, documented by preliminary laparoscopy; Idiopathic infertility for at least 4-6 years (variable based on patient's age).

*III. IVF-ET attempt in connection with diagnostic laparoscopy* (in women with normal hysterosalpingography and insufflation test): Ovulatory defects successfully treated for at least 6 cycles; cervical hostility (previous phlogosis; iatrogenic; immunological; etc.); slight seminal fluid defects: Possible idiopathic infertility.

### Genetic screening for IVF-ET patients

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Although it is not customary for a couple undergoing investigation and therapy for sterility to be screened for genetic defects, such an approach might be envisaged in IVF-ET. There are two reasons for this: 1) The financial burden of the procedure for the parents, the state, the health-insurance schemes, etc. which would be even more serious if a handicapped child was produced. 2) The psychological burden for the couple and the treating physicians.

Selection of patients for IVF-ET should consider the following genetical aspects: 1) Seriously debilitating multifactorial traits should not be present in the couple or in more than one close relative. The same applies to dominantly inherited diseases. 2) IVF-ET should not be performed in a couple who have already had a child with an autosomal recessively inherited disease, or in consanguineous marriages, when such a disorder is present in a close relative of both. 3) In a woman with a brother, maternal uncle or grandfather with a serious X-chromosomal recessively inherited disease IVF-ET should only be performed when reliable tests for carrier detection exist. 4) Drug users and alcoholics should not be granted IVF-ET because of possible damage to the fetus. For the same reason, epileptic women should be changed to the least teratogenic antiepileptic drug if possible, before IVF-ET is initiated. 5) To rule out chromosomal abnormalities induced by the procedure prenatal diagnosis should be offered to women undergoing IVF-ET.

## Genetic aspects of human infertility

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The average incidence of chromosome abnormalities from samples of infertile males has been established at approximately 5%, of which 4% are sex chromosome abnormalities and 1% autosomal abnormalities. Variations in the frequencies among different samples are probably due to bias in ascertainment. The autosomal abnormalities consist mostly of balanced translocations, which are found with a frequency of 9%. Most translocations are Robertsonian ones and most of them are familial. The reasons why a balanced translocation interferes with the normal meiotic process were discussed. The effects on female fertility of numerical and structural aberrations of the X chromosome were discussed with special attention to the deficiencies of the short and the long arm of the X chromosome. Several of these deficiencies are secondary to X/autosome translocation transmitted by the mother of the probands. It is concluded that the function of the abnormal X chromosome is likely to be correlated with a lack of gamete production, rather than the loss of specific segments.

## Stimulation and monitoring of follicular maturation

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Although the first birth after IVF-ET (Stephens and Edwards 1978) was achieved by means of an oocyte matured in a natural cycle, nowadays all groups working in this field recognize the importance of ovarian stimulation in order to collect more mature oocytes. But beyond the monitoring of the cycle, the stimulation itself has several intrinsic problems: It has been demonstrated that the different methods of stimulation reduce notably the proportion of follicles with normal steroid concentration. Furthermore, the results of different research groups have pointed out the difficulties of employing a standard protocol. In fact, it seems necessary that a special and adequate treatment protocol should be applied to each patient. Of the different methods in use, it is evident that at the moment Clomiphene citrate, frequently combined with HMG and HCG, is the most used pharmacologic agent for induction of ovulation. The monitoring of the preovulatory phase is done nowadays by echography, by hormone assays and by evaluation of the cervical mucus.

In view of the many methodological problems encountered in the application of each one of these methods for monitoring the cycle, a successful protocol should include a synthesis of information derived from all of them. These methods are keys to obtaining an acceptable percentage of successes in this new and sophisticated technique.

## Aspiration of oocytes through ultrasound guidance

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Repeated microsurgical operations have been considered for a long time to be hazardous and this opinion may even, to a certain extent, be applicable to repeated laparoscopies in an IVF/ET program. In an attempt to minimize the operative risks connected with repeated oocyte aspiration, we have developed a technique for transvesical ultrasound guided aspiration on an outpatient basis. The aspirations

are performed under either local or light general anesthesia, utilizing a real time, linear array, standard ultrasound apparatus (Hitachi EUB 25M). Today, this equipment is commonly used for various obstetrical and gynecological purposes in many departments. On the short side of the transducer a small plastic steering device is attached for the puncturing needle. The predetermined puncturing angle at which the needle will enter the sound field is fixed to 60° with respect to the transducer face. The needle direction is indicated by a dotted line on the monitor and this line should pass through the maximal diameter of the follicle to be punctured. A 23 cm long siliconized steel needle (inner diameter 1.6 mm, outer diameter 1.8 mm) connected by teflon tubing to a sampling flask and a vacuum pump (Craft Suction Unit Rocket Ltd, London) has proved optimal although needles with inner diameters down to 1.0 mm may be used. Drilling shallow scores at the top of the needle improves the visualization and gives a more optimal positioning of the needle tip within the follicle. On the 100 patients treated to date the transvesical puncture technique has not caused any serious damage. Slight hematuria lasting for 1-2 days after the aspiration has been noted in 4 patients. The oocyte retrieval rate on a patient basis is presently 87%, which can be compared with a success rate of 85% when laparoscopies are performed on our patients. The ultrasound-guided technique appears to be especially useful in cases where severe adhesions make laparoscopic visualization of the ovaries impossible. The avoidance of exposure of the ovaries to CO<sub>2</sub> may also be of advantage. Furthermore, the procedure seems less stressful to the patient when performed as an outpatient service. Utilization of an outpatient service reduces the costs considerably and makes the procedure more flexible regarding choice of optimal time during the day for the punctures. Hitherto, 3 clinical pregnancies (1 twin pregnancy) and 8 'biochemical' or 'suspected' pregnancies have been registered.

## Aspiration of oocytes by laparoscopy

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Technical problems of laparoscopic oocyte aspiration are today no longer a matter for discussion, although minor differences in the practical work between the IVF-centers still exist.

1) Pneumoperitoneum can be built up with CO<sub>2</sub>; nitrogen gas does not improve fertilization rate. 2) Outer and inner diameter of 1.2 mm with a continuous teflon tube is the most widespread variation, and delivers undamaged oocytes. 3) Vacuum strength can vary between 100 to 300 mm Hg without influence on the recovery rate. 4) Site of puncture depends on the size and ripeness of the follicle; the thinner the follicular membrane, the more the follicle should be evacuated by a tangential approach.

A point of controversy is still whether the oocytes should be obtained in a natural cycle or after ovarian stimulation (clomiphene, gonadotropins). It also seems not to be clear whether every visible follicle or only preovulatory follicles should be aspirated. The presented results (after clomiphene and after combined clomiphene/HMG stimulation) show that the recovery rate (oocytes per aspirated follicles) increases with increasing follicle size. The fertilization rate is nearly constant between follicle sizes of 3-10 ml follicular fluid, and decreases for smaller and larger follicles. From the 166 transferred embryos 59 developed from oocytes which were recovered from follicles smaller than 4 ml (<2 ml:16; 2 ml:20; 3 ml:23). Two pregnancies (out of 20) resulted from oocytes out of 2 ml follicles. We therefore conclude that it is worthwhile to puncture every follicle which can be reached.

## Acrosome reaction of human sperms in vitro

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In this study we used the triple stain procedure of Talbot and Chacon (J. exp. Zool. 215 (1981) 201) to estimate the acrosome reactions during incubation of the spermatozoa in capacitating conditions. Simultaneously the fertilizing ability of those sperms in the zona free hamster egg test (H.P.T.) was determined. We used fresh sperms of the sperm bank of the fertility unit (Dept. of Gynecology, Basel). During the incubation the percentage of live acrosome-reacted sperms usually increased. Peak levels were reached after 3–7 h, but on two occasions no peak levels could be detected. In these samples the number of acrosome-reacted sperms remained at a level of 10%. In other cases peak levels reached 30–70%. An increase in the number of acrosome-reacted sperms coincided with an increase in the penetration rate in the H.P.T. We found high values after 3–7 h of incubation, but very low or even zero values when the sperms were added to the eggs directly after washing away the seminal plasma. Although considerable numbers of live reacted sperms were still present after 20 h of incubation, the penetration rates in the H.P.T. decreased. This suggests that the life span of sperms is limited once they have undergone the acrosome reaction.

## Idiopathic infertility and IVF-ET

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A multi-disciplinary study revealed in 1982 that women suffering from idiopathic infertility are different from women of a control-group in 3 aspects: 1) They complain more of dysmenorrhea, 2) They have a lower self-esteem (a big difference between self image and desired image), 3) They are more compulsive and obsessive than the controls. Our simplified interpretation is: Insufficiency feelings in these patients are derived from a suppression of personality development (by family and/or others). Compulsive attitude attempt to avert feelings of guilt and insufficiency. Dysmenorrhea is a conversion symptom reflecting the ambivalent desire for a child (the conscious wish for a child is opposed by a subconscious fear of recurrence of the own family-child relationship). We had 7 women with idiopathic infertility in our IVF/ET program. 6 of them had the above mentioned symptoms, 2 of them experienced unexplained fever after ET and none of them became pregnant in the treatment cycle. The patient without symptoms became pregnant and has borne a healthy baby. 2 of the patients with symptoms became pregnant later, after a psychological treatment. In view of the above findings, we now prefer a psychological treatment for patients with idiopathic infertility. We even consider additional psychogenic infertility factors in patients with tubal sterility when repeated trials with IVF and ET have failed. In one case with tubal sterility 5 nice-looking embryos were transferred during 3 cycles without success. After having resolved her partner problems with the help of psychological counseling the patient conceived in her 4th trial of IVF, after transfer of only 1 embryo in the pronucleate stage.

## Psychosomatic counseling of couples involved in an IVF-ET program

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We asked 374 of the childless couples in our clinic how they felt about IVF. Of these couples, 43.9% showed a very

positive attitude and 34.2% a moderately positive attitude, whereas 11.2% of the couples were more or less against it and 10.7% rejected the method categorically. On the basis of this generally positive response, the IVF method is gradually becoming a matter of clinical routine. Our experience has shown that psychosomatic counseling of the couples involved is essential, all the more so since both partners agree that the psychic burden during the IVF process is greater than the somatic one. In view of possible contraindications such as psychosis, severe neurotic depression etc., we attach great importance to the individual counseling of both partners. A confidential and above all frank doctor-patient relationship at an early stage is instrumental in helping these couples to overcome or even avoid possible psychic side-effects.

## Computerized analysis of factors affecting successful embryo transfer in humans

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Our program of IVF and ET has been successfully in operation since November 1981. Our clinical experience is based on three different types of IVF centers which we have organized: 1) Big hospital/university clinic: Laparoscopy, IVF-laboratory in the operating theatre, ultrasonic department, hormone laboratory (radioimmunoassays); 2) Gynecological private office: Hormone laboratory (hemagglutination tests for E<sub>2</sub>, LH) ultrasonic examination, IVF-laboratory in the office; Laparoscopy in a hospital and transport of eggs in follicular fluid to the laboratory. Alternatively: ultrasonic guided follicle puncture under local anesthesia in the office. ET as an outpatient procedure (bed-rest max. ½ h); 3) Different gynecologists and hospitals cooperate with specialized IVF-laboratory based on egg and embryo transport as in system 2).

A computerized analysis of all IVF data from systems 1) and 2) was carried out on 233 treatment cycles for 170 patients (total number treated in 1982). There was no statistical difference between the results of systems 1) and 2). The pregnancy rate was 10% per treatment cycle in both systems and 17% (system 1) or 19% (system 2) respectively. The results of system 3) were not satisfactory, therefore this system was abandoned (no pregnancy from 20 laparoscopies in 1982). The age range of all patients was 20–44 years. There was neither a significant difference of follicle numbers, recovered eggs, fertilization and pregnancy rates between different age groups, nor any significant correlation of the age of the patients with the number of follicles found at laparoscopy, the recovery rate of mature eggs and the fertilization rates. The age distribution was  $30.75 \pm 4.7$  years in the not-pregnant group and  $29.56 \pm 4.7$  years in the pregnant patients.

In the pregnant group we found embryos to be in a 4-cell stage at a mean of 42.28 h, and 6–8-cell embryos at 43.33 h, whereas when embryos failed to establish pregnancy they had 4 cells at 44.57 h and 6–8 cells at 45.1 h. The implantation rate was only 11.7% with single embryos, significantly different from twin-transfers where a pregnancy rate of 30% was achieved. The transfer of triplets gave a pregnancy rate of 36.3%. There was no significant difference in the pregnancy rate using 3 different ET-catheter types. Ultrasonically guided follicle aspiration under local anesthesia enabled us to perform the whole treatment procedure of monitoring, ovum harvest, IVF and ET as an outpatient-office procedure. Our first attempts at ultrasonically guided follicle aspiration resulted in one normal ongoing pregnancy out of 5 ETs. 20 eggs were recovered from 22 follicles but only 9 of them were fertilized.

The authors wish to emphasize that their work was based on years of clinical experience in this field. Oocyte handling, fertilization and culture should always be restricted to specially trained staffs. We are therefore still a long way from IVF becoming a widely-used routine office procedure.

## Results of the Erlangen IVF program

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Since January 1981 the Universitäts-Frauenklinik of Erlangen has offered extracorporal fertilization to suitable patients as a new means of sterility treatment. The description of the follicle puncture technique, as employed in our program, is the main subject of this report. A summary of the total results is also given.

The current state of oocyte recovery rate was demonstrated by data from a consecutive series of patients. The instruments and technique of follicular puncture were described in detail. 30 women were stimulated with clomid and HCG, and 78 large follicles (vol. >5 ml) were found during laparoscopy. 77 (70 + 7) oocytes were recovered by follicular aspiration and rinsing, respectively, and of these 53 were fertilized. 47 cells cleaved regularly and were transferred to 21 patients. The oocyte recovery rate of almost 100% cannot be further improved. This perfection of results was obtained through many small steps of improvement over a period of 2 years. Reasons possibly responsible for this development were discussed.

Finally a summary of the results of the Erlangen IVF program (Jan 81-Jan 83):

No. of laparoscopies with follicular aspiration	169
No. of embryo transfer (patients)	130
No. of clinical pregnancies achieved	18
No. of children born	6

## Committee for bioethics in the handling of human gametes and embryos established by the Swiss Academy of Medical Sciences

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In 1982 the Swiss Academy of Medical Sciences followed the proposal of medical doctors, gynecological hospitals

and research foundations, in establishing a new committee for bioethics in the handling of human gametes and embryos. The membership of the committee consists of experts in the field of IVF and ET for the treatment of human infertility: scientists, medical doctors from different disciplines, and also philosophers, including a woman, a lawyer and a moral theologian. The principal aims are to elaborate the guidelines for IVF and ET for medical purposes and for experiments with gametes and embryos. These guidelines are to lay down the boundaries by which all activities become unethical and not compatible with Swiss law. A second aim is to effect a quality control of the activities carried out in this field through direct contact with the persons involved. Above all, it is intended to appeal to the sense of responsibility of the persons concerned. Furthermore, the committee wishes to place itself at the disposal of all doctors and scientists interested in the relevant problems, to serve as a source of information on specific questions. The guidelines elaborated by the committee will be published by the Swiss Academy of Medical Sciences.

## In vitro fertilization and embryo transfer 1983 and tomorrow?

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More than 200 births have already been achieved by IVF and ET in various parts of the world, and many more pregnancies are still in progress. Clinical assessment of the newborn children gives no evidence of increased risk of genetic or developmental damages.

Many potential developments can be expected in the field: Cryoconservation of the fertilized ova, pre-implantation genetic analysis, genetic engineering on fertilized ova for diagnosis and treatment of genetic disease will probably be achieved in the future. Cloning of human cells and 'improvement' of genetic potentialities are among the main dangers to be avoided.

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