In conclusion, it should be emphasized that simplifications of the technique are only justified if they do not negatively interfere with the success rate which in almost all groups is still unacceptably low, at least when based on total patient numbers. It is, however, our belief that IVF/ET will appear soon as an out-patient procedure in many countries. Three term pregnancies have already been achieved by our own out-patient procedure according to the outlined simplifications.

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Multiple pregnancies in gonadotropin-stimulated cycles after human in vitro fertilization (IVF) and embryo replacement

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Key words. In vitro fertilization; embryo replacement; multiple pregnancies; sterility treatment; follicular puncture.

Introduction

The fertilization process, which till recently took place in the obscurity of the mother's womb in mammals, including the human and other primates, can be performed nowadays extracorporally. The current developments have dramatically testified that external fertilization, a phenomenon well known in fish, can now be regarded as a routinely applicable method of assisted fertilization in man. After various indications oocytes are aspirated from the follicles, inseminated in vitro with husband's sperm and replaced into the mother's womb after reaching a normal-looking four-cell stage. Various methods of preparing the patients for this technique have been applied[6, 10, 11]. It is the aim of the present paper to report on a large number of follicular punctures, in vitro fertilizations and embryo replacements in gonadotropin stimulated cycles. Multiple pregnancies occurred in 50% of the pregnancies. Is this a desirable or a necessary side effect of this technique of sterility treatment?

Material and methods

1) Hormonal preparation

In order to achieve a higher frequency of aspirated oocytes per cycle, we have been stimulating our patients, since September 1982, with Clomid/hMG/hCG, Clomid/hCG or hMG/hCG alone. It is known that in the natural cycle hMG suppresses an endogenous LH rise. If a spontaneous LH surge did not occur human chorionic gonadotropin (hCG) was used and pelviscopy for follicular aspiration was scheduled 36 to 38 h after the administration of 10,000 IU hCG. hMG was given individually according to the daily measured estradiol response and follicle sizes measured by ultrasound scans. Some patients were stimulated with 150 mg Clomid from day 3 to 7, some from day 4 to 8 or from day 5 to 9 depending on the length of the previous cycles, recorded by the patient herself. The development of an estradiol plateau following a continuous increase in correspondence to the num-

ber of dominant follicles indicated the time of hCG application.

2) Patient selection

Patients of the following categories, occasionally with overlapping indications, were considered suitable for the program:

Category 1: Tubal pathology

Category 2: Decreased sperm factors (genetic screening advisable)

Category 3: Idiopathic sterility

Category 4: Sperm antibodies in serum or seminal plasma

We only accepted couples who fulfilled the requirements listed below:

- existence of uterus and at least one ovary in the woman,
- sperm count with certain minimal criteria: number > 5 mill., motility $> 30\%$, morphology $> 30\%$ normal, neither mycoplasms nor aerobic or anaerobic bacteria.

3) Monitoring of follicular maturation in the stimulated cycles

To determine the time of ovulation, serum-LH levels were measured by a rapid 1-h LH-radioimmunoassay (RIA) from Serono, which was modified by abbrevation of the incubation time and elevation of the incubation temperature[7]. Estradiol (E_2) was determined with a 3-h Travenol-RIA directly from the sera without extraction. Serum-progesterone (P) levels were measured with the Behring RIA kit using rabbit antiprogesterone serum in a 6-h assay.

Follicular development was further monitored by observing the cervical mucus rheological characteristics with the extended cervical score and serial ultrasonographies. After an initial ultrasonography on day 2, depending on the hormonal increase of estradiol, daily ultrasound scans of the follicles were performed in order to observe the leading follicle reaching 1.8 to 2 cm in diameter. As soon as the extended cervical score had reached 15 points and the serum 17-β-estradiol levels had increased to a minimum of 310 pg/ml for each follicle larger than 1 cm in diameter, hCG was applied.

Pelviscopy in patients with spontaneous LH-rises was carried out 30 h after the retrospective estimated time of the surge.

4) Follicular puncture via pelviscopy

Follicular puncture was carried out via pelviscopy in the Trendelenburg position under general anesthesia. A 100% CO_2-pneumoperitoneum was produced and electronically controlled[9]. In many cases adhesiolyses and ovariolyses had to be performed before the ovaries were ready for the puncture.

For the follicular puncture a teflon-coated puncture cannula with an inner diameter of 1.2 mm and an outer diameter of 1.8 mm, which is introduced through a trocar sheath of 2 mm outer diameter after retraction of a mandrin, was applied. Follicular fluid was aspirated under a vacuum of 120 mm Hg controlled by the Labotect-Vacuum-Aspirator with a footswitch. To collect the oocytes, we used 50 ml Falcon tubes, which were kept in 37 °C isothermic containers and were passed directly for screening to the tissue culture laboratory adjacent to the operating theatre. As soon as an oocyte was found, the follicle puncture cannula was retracted from the follicle. In negative cases the follicle was rinsed with heparinized culture medium containing 5% maternal serum.

Parameters observed at the time of follicle puncture were:

- The volume of follicular fluid (ml).
- Classification of oocytes according to three grades of maturity depending on the relationship of the oocyte to the cumulus mass. Fully mature oocytes have expanded cumulus cells with a dispersed corona. The oocyte is clearly visible (Grade +++). Immature eggs have adherent cumulus cells with a dense corona (Grade +).

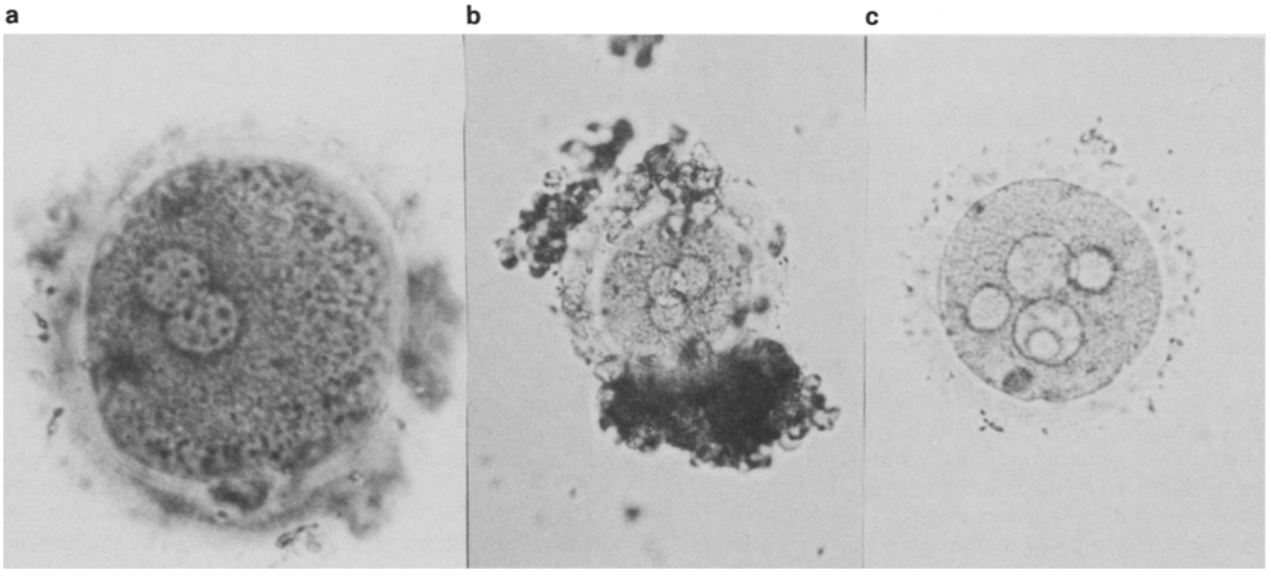


Figure 1. Human embryos 16–18 h after insemination showing two and more pronuclei.

5) In vitro fertilization and embryo culture

The egg was incubated either in HAM's F-10 or Menezo's medium with 7.5% maternal serum for 6 h at a constant temperature of 37°C in 5% CO_2, 5% O_2 and 90% N_2. Semen from the husband was obtained 1–2 h prior to insemination. The oocytes were inseminated with 1×10^5 motile spermatozoa/ml 6 h after collection. 16–20 h after insemination the fertilized egg was transferred to culture medium containing 15 vol.% inactivated patient's serum. Incubation was then allowed to continue in the automatically gas-controlled desiccator (Labotect, Göttingen, FRG). Fertilization had occurred if 16–20 h after insemination two pronuclei were clearly visible. The appearance of more than two pronuclei was the visible sign of a polyspermic reaction which had led to a polyploid oocyte (fig. 1). Approximately 42 h after insemination the embryos were examined for signs of cleavage (2- to 8-cell-stage).

6) Embryo replacement

In cases where cleavage had occurred, the embryo was replaced into the uterine cavity 48 h after insemination. At that time it has reached the 4- to 8-cell-stage. Experience has shown that embryo replacement requires a well-trained team of two persons. The embryo replacement is performed in a knee-chest position, so that the patient is facing head-down. The patient does not receive any premedication. The embryo is replaced with the so called 'Kiel-Model-Catheter' (fig. 2). With the aid of a tuberculin syringe the embryo is drawn up into the catheter from a center-well culture dish, where the embryos were collected just previously. Inside the catheter the embryos are separated by air from the tip of the catheter and from the following medium. Then the cervix is exposed by a speculum and cervical mucus is removed with saline. We try not to use a tenaculum. The catheter is introduced beyond the internal os, and advanced into the uterine cavity close to the fundus. With the tuberculin syringe the embryos are expelled with as little fluid as possible (5–20 µl). After a check up of the catheter under the stereomicroscope to ensure that the embryo has been transferred, the patient has to stay in her position for at least 3 h. In cases where the catheter does not pass the cervix, the cervix is grasped by a tenaculum and/or a metal catheter plus mandrin is introduced through the cervix to insert the catheter.

Results

Between September 1982 and October 1984, 313 pelviscopies with the aim of follicle puncture, ovum pick-up, in vitro fertilization, and embryo replacement were performed. According to the various stimulation schemes (Clomid/hMG/hCG; Clomid/hMG or hMG/hCG) different groups were evaluated. Tables 1–5 summarize the results of that period. A successful oocyte collection was achieved in 93.0% of pelviscopies with a total of 3.2 oocytes per patient. The fertilization rate was 50%. 193 embryo replacements were performed with an average number of 2.4 embryos per patient. Out of 28 pregnancies 10 are completed, 10 are continuing, one led to a miscarriage, and seven replacements resulted in a so-called biochemical pregnancy. This is an overall pregnancy rate of 14.5% per embryo replacement and 9.0% per pelviscopy performed.

Gonadotropin stimulation resulted in a sufficient increase of estradiol in the majority of patients. Patients not showing the desired endocrinological effects and

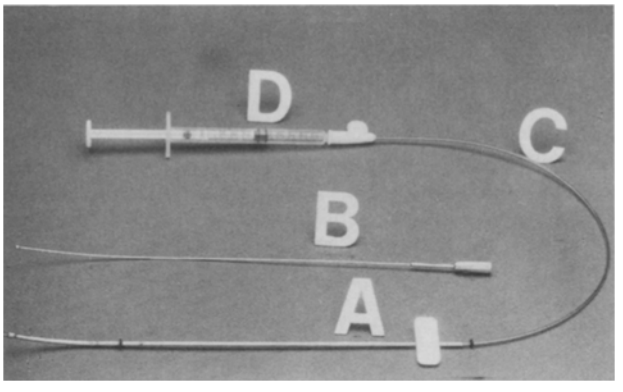


Figure 2. Embryo-replacement set KIEL (Teflon catheder with a rounded up tip and a metal sleeve with mandrin). Mostly the teflon transfer catheder (Vygon) alone is applied. In cases with a rigid cervix the metal sleeve is used.

Table 1. Pelviscopies and egg recovery in patients from September 1982 to October 1984

Type of stimulation	Pelviscopies		Patients with oocytes		Patients without oocytes		Oocytes	Oocytes/patient
	n	(%)	n	(%)	n	(%)	n total	n
Clom./hMG/hCG	213	(100)	193	(90.6)	20	(9.4)	570	2.7
hMG/hCG	87	(100)	85	(97.7)	2	(2.3)	409	4.7
Clom./hCG	13	(100)	13	(100)	0	(0.0)	26	2.0
n (%)	313	(100)	291	(93.0)	22	(7.0)	1005	3.2

Table 2. Fertilization rates and embryo replacements in patients from September 1982 to October 1984

Type of stimulation	Oocytes		Oocytes fertilized		Patients with oocytes		Embryo replacement		Embryos/ patient	Pelviscopies		Embryo replacements	
	n	(%)	n	(%)	n	(%)	n	(%)		n	(%)	n	(%)
Clom./hMG/hCG	570	(100)	260	(45.6)	193	(100)	122	(63.2)	2.1	213	(100)	122	(57.9)
hMG/hCG	409	(100)	189	(46.2)	85	(100)	65	(76.5)	2.9	87	(100)	65	(74.7)
Clom./hCG	26	(100)	12	(46.1)	13	(100)	6	(46.1)	2.0	13	(100)	6	(46.1)
n (%)	1.005	(100)	461	(45.9)	291	(100)	193	(66.3)	2.4	313	(100)	193	(61.7)

Table 3. Pregnancy rates per pelviscopy in patients from September 1982 to October 1984

Type of stimulation	Pelviscopies		A Elevated β-hCG; no fetal development		B Miscarriage		C Ongoing pregnancies		D Completed pregnancies		n (%) A–D	B–D
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)		
Clom./hMG/hCG	213	(100)	4	(1.9)	1	(0.5)	1	(0.5)	5	(2.3)	11 (5.2)	7 (3.3)
hMG/hCG	87	(100)	2	(2.3)	1	(1.1)	9	(10.3)	3	(3.4)	15 (17.2)	13 (14.9)
Clom./hCG	13	(100)	–	(–)	1	(7.7)	–	(–)	1	(7.7)	2 (15.4)	1 (7.7)
n (%)	313	(100)	6	(1.9)	3	(1.0)	10	(3.2)	9	(2.9)	28 (8.9)	21 (6.7)

Table 4. Pregnancy rates per replacement in patients from September 1982 to October 1984

Type of stimulation	Embryo replacements		A Elevated β-hCG; no fetal development		B Miscarriage		C Ongoing pregnancies		D Completed pregnancies		n (%) A–D	B–D
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)		
Clom./hMG/hCG	122	(100)	4	(3.3)	1	(0.8)	1	(0.8)	5	(4.1)	11 (9.0)	7 (5.7)
hMG/hCG	65	(100)	2	(3.1)	1	(1.5)	1	(13.8)	3	(4.6)	15 (23.1)	13 (20.0)
Clom./hCG	6	(100)	–	(–)	1	(16.7)	–	(–)	1	(16.7)	2 (33.3)	1 (16.7)
n (%)	193	(100)	6	(1.6)	3	(1.6)	10	(5.2)	9	(4.7)	28 (14.5)	21 (10.9)

Table 5. Pregnancy rates in patients from September 1982 to October 1984 with different modes of stimulation

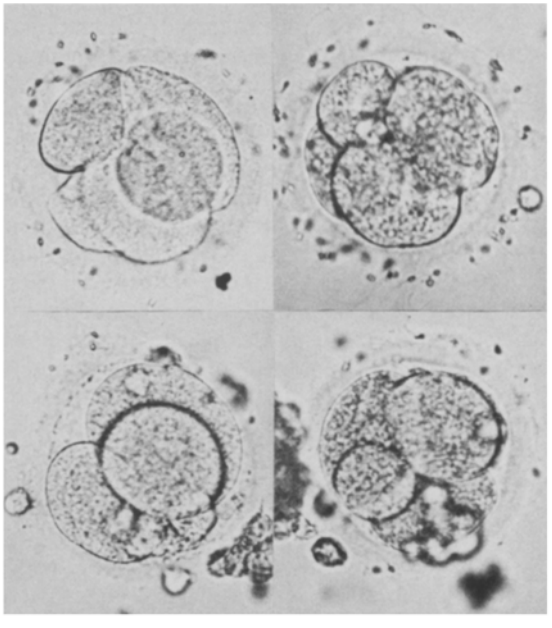
	Pelviscopies n = 313 (100%)	Embryo replacements n = 193 (100%)
Total pregnancies n (%)	28 (9.0)	28 (14.5)
Ongoing/completed pregnancies n (%)	19 (6.1)	19 (9.9)

continuous follicular growth were dismissed from the program and asked to return in a later cycle. However, in patients not previously known, follicular punctures were carried out even in suboptimal cases.

Out of 20 ongoing pregnancies observed up to September 1984, nine were multiple pregnancies (45.0%). Figure 3 demonstrates our first delivery of triplets and the four embryos replaced.

Discussion

Multiple pregnancies do occur in IVF-embryo-replacement programs. The perinatal mortality for twins is known to be 4–6 times higher compared to the singleton perinatal mortality. For triplets and further multiples this ratio increases further. Multiples are not intended, as they increase the obstetrical risk for the patient. Nevertheless, one set of quadruplets has been delivered in Australia, and several successfully terminated pregnancies of triplets and twins have been reported. In addition, it must be considered that a high ovarian stimulation which will lead to the aspiration of more than five eggs will cause a follicular dischrony and disturb the corpus luteum function and implantation. For this reason a mild ovarian stimulation which leads to an aspiration rate of up to five eggs should be preferred. The replacement of three to four eggs is regarded as being optimal at the present time. Taking into consideration that higher pregnancy rates were achieved after transferring multiple embryos (helping phenomenon) into the uterine cavity[1], we have transferred up to five embryos per replacement, if this is desired by patient. Kerin et al.[5] reported seven multiple pregnancies out of 20 in Adelaide, Australia, and con-



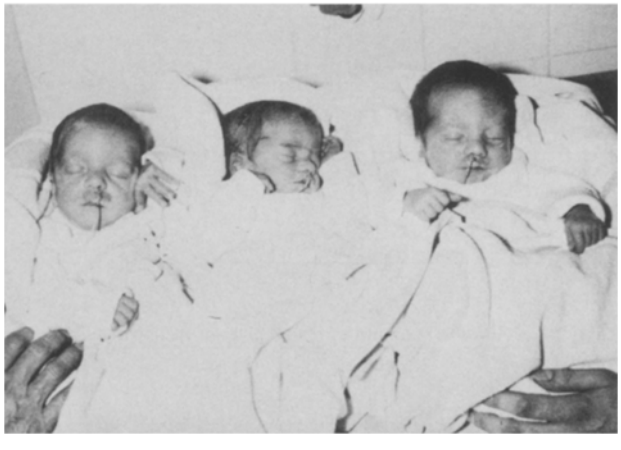


Figure 3. Four 4-cell stages after in vitro fertilization that were replaced in a patient's uterus and resulted in a triplet pregnancy that was terminated in 34th week by Caesarean Section. Three healthy boys were delivered.

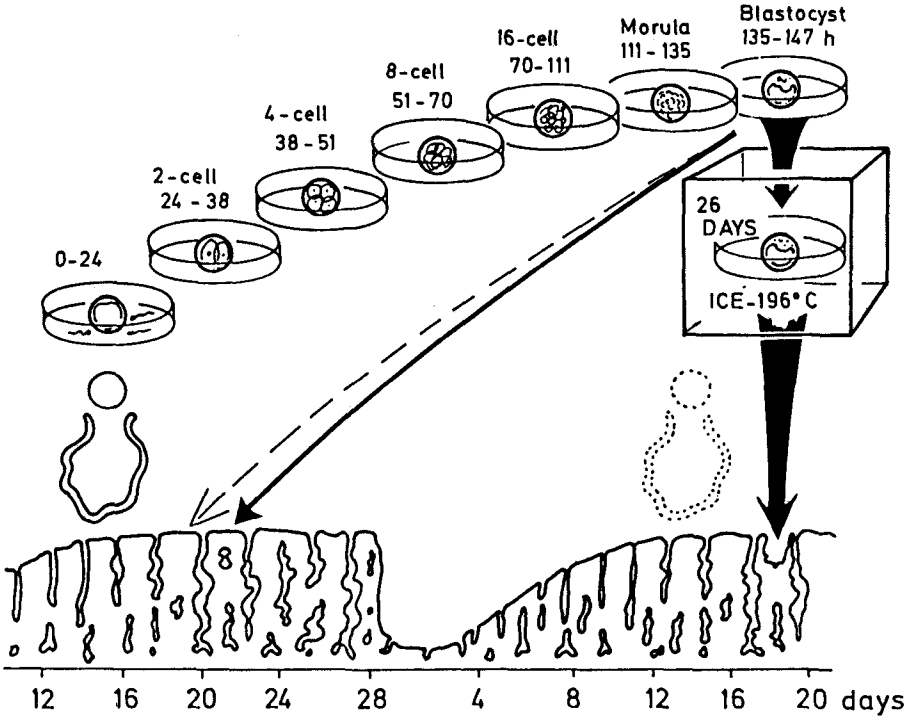


Figure 4. Embryo replacement in an unstimulated cycle after freezing and thawing (a thought).

sidered reducing the number of embryos replaced from originally 4–2 to prevent further multiple pregnancies.

Our two triplet pregnancies have caused only minor obstetrical problems. Both had to be delivered in the 34th week of gestation by caesarean section, and three boys and three girls were delivered. In contrast, our monochorionic biamniotic twins caused severe obstetrical problems. The patient had a rupture of the membranes of one twin in week 14. Increased leucocytes and uncontrollable labor terminated the pregnancy in week 29. Two girls (< 1000 g) were delivered by caesarean section and were raised with difficulty owing to their premature delivery.

The variability in an in vitro fertilization and embryo replacement program is considerable; however, the IVF guidelines prohibit experiments to test the different variables. The limited availability of fertilizable oocytes and embryos, as well as a policy of complete replacement of all embryos, has made it necessary to develop a procedure based on evidence. Thus the stimulated cycle has proved to be more useful for the harvesting of oocytes than the unstimulated cycle. Pure gonadotropin stimulation seems to be superior to Clomid/hMG/hCG treatment.

Several authors[2–4, 6] have given advice on the careful handling of embryos. Their idea of supplementary in vitro incubation to mature the immature oocyte proved to be successful in our material. Some eggs were incubated for an additional 10–15 h before insemination.

We cannot confirm that the exposure to light of the oocyte and the early embryo in order to photograph them precludes cleavage. Photos were taken with as little light as possible and embryos were handled with great care. They cleaved in a normal way and were replaced. Only the highest grade of internal quality control guarantees optimal results. One of our criteria is that all culture media must support cleavage of early mouse embryos.

Compared to our unsuccessful period[8], it became obvious that only a program performed with total dedication of clinicians and laboratory staff can lead to success. Although it is our primary aim to help infertile couples, the program offers the opportunity to investigate many aspects of reproduction, such as the ultimate details of follicular maturation, sperm-oocyte interaction, the development of two or more pronuclei, chromosome patterns of early cleavage stages, etc.

The application of gonadotropins only resulted in the highest pregnancy rates. However, in hMG stimulated cycles corpus luteum deficiencies became apparent. Previously no hormonal support of the corpus luteum phase was given. At present progesterone and/or repeated hCG is applied. To improve the physiological pregnancy rate of about 25% by the in vitro fertilization and embryo replacement program, it is necessary to give gonadotropin support and to replace up to four embryos, accepting the side effects of multiple pregnancies. Nevertheless, the ultimate aim is to increase singleton pregnancies.

With an increasing success rate for freezing human embryos, the concept of replacing an embryo after deep freezing in a consecutive cycle which is not treated by stimulation, becomes more and more attractive. The successful pregnancy achieved in Calcutta, India, by the work of the late S. Mukherjee in 1980 resulted in a living girl now 4 years of age. The use of this procedure must still be agreed upon by us all, but it does suggest that the concept illustrated in figure 4 may increase the pregnancy rates achieved.

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Psychosomatic counseling of couples involved in an in vitro fertilization (IVF)–embryo transfer (ET) program

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Key words. In vitro fertilization; embryo transfer; psychosomatic counseling.

The response of the general public to the first successful extracorporal fertilizations showed on the one hand admiration for the medical progress demonstrated, but on the other hand scepticism to the point of rejection. The objections were mainly because of fears that the use of procedures which had become technically feasible in medicine could not be controlled. The fear of possible abuse was one cause for concern; it is indeed clearly necessary that an ethical framework should be developed.

From the psychosomatic point of view, we were interested in knowing the personal opinions of our childless couples (women patients) about extracorporal fertilization[1,2]. In 1982, therefore, even before our first extracorporal fertilization, we asked the couples who desired a child about their opinions on the 'Test-Tube-Baby'. They had to check four different possibilities on a questionnaire:

1) Positive attitude
2) Moderately positive attitude
3) Moderately negative attitude
4) Negative attitude

43.9% of the couples desiring a child showed a very positive attitude towards IVF and 34.2% a moderately positive one. 11.2% of the couples were more or less against it, and 10.7% rejected the method categorically. We may consider the opinion of the patients concerned to be unmistakable. 78.1% of our childless couples regarded this method in a positive manner; in other words, three out of four couples with a desire for children would take into consideration IVF if this procedure were indicated.

A further investigation was concerned with the presence of psychic and psychosomatic symptoms in couples who desired extracorporal fertilization. These findings led to the result that, by psychological standards, the average couple wanting IVF does not represent a peculiar pathological group of patients among couples desiring a child. The emphasis must be placed upon the word 'average'. There may be some cases of patients for whom IVF would be contraindicated for psychosomatic reasons.

Contraindications in view of psychosomatic problems:
- in case of psychosis in one of the partners,
- in case of severe neurotic depression in one of the partners,
- if one partner shows an ambivalent desire for a child,
- it is hoped that the desired child will sustain the partnership,
- in case of functional (idiopathic, psychogenic) sterility.

In order to get criteria for useful psychological guidance of couples for IVF, we investigated 20 couples in detail by means of interviews and questionnaires. From this still relatively small amount of experience we have collected mostly phenomenological data concerning the psychic stress involved. Thus first guidelines for the assistance of patients have been prepared.

All our patients agreed with the statement that the psychic burden during the IVF process is greater than the somatic one. Particularly the great tension during the period between hope and reality was stressed by nearly all patients, though they worked it out in individually different ways. Therefore we attach great importance to the individual counseling of the patients[3]. The couple should not have more than one or two doctors as counselors. A