Clinical and logistical aspects of in vitro fertilization treatment

An analysis of a transport IVF programme

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Klinische en logistieke aspecten van in-vitro fertilisatie

Een analyse van een transport IVF programma

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. P.W.C. AKKERMANS M.A.
EN VOLGENS BESLUIT VAN HET COLLEGE VOOR PROMOTIES
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 11 JUNI 1997 OM 11.45 UUR

DOOR

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GEBOREN TE WEESP

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The publication of this thesis was supported by Organon BV.

1997 by Jan Roest
ISBN 90-9010640-5

Printed by Van den Berg & Versluijs BV Dordrecht

Cover: Aq'aba, wooden doll used as fertility symbol by the Ashanti people from Ghana to ascertain the birth of healthy children.

Aan Adri

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General introduction

In vitro fertilization and embryo transfer (IVF/ET), the culture of aspirated oocytes and spermatozoa in the laboratory followed by transcervical embryo transfer, was originally used as a treatment for infertility resulting from impaired function of the fallopian tubes. A few years after the introduction of IVF/ET the indications for treatment included infertility caused by endometriosis, male factors, ovulation disorders and unexplained infertility (1). Although IVF has now become an accepted treatment for infertility, there are still several contentious issues in the application of assisted reproductive technologies (ART) (2). It has been argued by World Health Organization authorities that IVF benefits only a small proportion of infertile couples, that it is expensive, and that it has serious health risks (3). In a WHO report from the Regional Office for Europe it was suggested that eligibility for IVF should be limited to women under 40 years of age, that the number of treatment cycles per woman should be limited, and that no more than three embryos should be transferred per treatment cycle (4). The increased incidence of triplet and higher-order pregnancies caused by ovulation induction and ART (5, 6), its associated increased medical risks (7) and high costs (8) have been reported. Apparently, some health authorities still consider ART controversial. This critical approach stresses the need for IVF programmes to raise their level of accountability.

In this thesis, the logistical and some clinical aspects of the IVF treatment as carried out in the transport IVF programme at the Zuiderziekenhuis, Rotterdam, in collaboration with the University Hospital Dijkzigt, Rotterdam, are analyzed. The aims of this thesis are: to determine the efficacy of a decentralized IVF programme with transport and satellite clinics, to further develop criteria for acceptance of patients, to develop a safer treatment strategy avoiding high-order pregnancies, and, where possible, to touch upon the issues mentioned above.

Logistical aspects of in vitro fertilization treatment

The argument that IVF benefits only a small proportion of infertile women (3) is not valid in The Netherlands. Only very few women do not benefit from insurance, and in practically all cases the cost of the first three IVF attempts is paid for by health insurance. Consequently, all patients with an indication for IVF can be offered three IVF attempts without major financial restrictions. The number of IVF laboratories in The Netherlands has been limited to 12 by government regulation. Initially, this led to long waiting lists for patients with an indication for IVF. Since the capacity of

the IVF laboratory is usually larger than the treatment capacity of the clinical team, transport IVF, with transport of aspirated oocytes from a transport IVF clinic to a central IVF laboratory was introduced to enhance the availability of IVF treatment to the public (9, 10). The logistical organization of the programme at the Zuiderziekenhuis was further developed by the introduction of satellite clinics where monitoring of the controlled ovarian hyperstimulation (COH) takes places. The cooperation between the transport centre and the satellite clinics offers the patients considerable advantages in terms of convenience, travelling time and expenses. In the first chapter of this thesis the results of this decentralized IVF programme are evaluated. Time spent on IVF treatment could be used productively in other endeavors and should be considered a component of economic costs. These indirect costs have been estimated to add $880 to the cost of an average cycle (11). Therefore, further simplification of the logistical organization by using a minimal monitoring schedule during COH makes an IVF programme even more accessible to patients, and lowers treatment costs even further. To achieve this, a minimal monitoring schedule for COH was introduced in the transport IVF programme at the Zuiderziekenhuis. The feasibility of this schedule is evaluated in chapter 2. Furthermore, the literature is reviewed with respect to the logistical advantages of the use of gonadotrophin-releasing hormone agonists (GnRH-a) in IVF treatment, and the experience with GnRH-a in the transport IVF centre is presented in chapter 3 .

Aspects of patient selection for in vitro fertilization

The decrease in fecundity with rising age is generally known. Schwartz and Mayaux (12) reported evidence for a decrease in fecundity after the age of 30. In IVF treatment the age of the female partner has been found to be an important determinant of success. Consequently, this has led to age limits for acceptance of patients in many IVF programmes. In the Netherlands the general opinion in IVF programmes is that patients aged 40 years and older should not be offered treatment. A WHO report (4) suggests to exclude patients aged over 40 years. However, the validity of such strict age limits can be questioned. Prestimulation tests proved to be better predictors of an individual patient's IVF performance than age (13-15). Chapter 4 is a review of the literature on the relation between age and IVF results. Chapter 5 is a study carried out to determine whether age or the ovarian response to COH is the better predictor for successful IVF treatment after the age of 40.

Male subfertility has become an accepted indication for IVF. However, in couples with an extreme male factor, semen parameters can be unacceptable for IVF because of expected low fertilization rates. For these couples intracytoplasmic sperm injection (ICSI) has proved to be an effec-

tive method of assisted fertilization (16) with a low fertilization failure rate (17). Failed fertilization in standard IVF is another reason to select patients for ICSI. The definition of failed fertilization used by Van Steirteghem et al. (18) was: the total absence of oocytes with two pronuclei 16-18 hours after insemination of a sufficient number of oocytes, or a fertilization rate of < 5% in several treatment cycles. This definition raises questions. When do we speak of a sufficient number of oocytes? Why should the fertilization rate be < 5%, or even ≤ 10% as mentioned by the same group (19) in a later publication? Preferably, IVF programmes should develop their own criteria for failed fertilization and assess the recurrence rate of this frustrating event in their own population. In the patient population of the transport IVF centre the treatment prognosis after poor fertilization in the first IVF cycle is assessed and recommendations for subsequent cycles are made in chapter 6.

Procedure-related clinical complications of IVF

In vitro fertilization is an elective procedure, and therefore the procedure-related complications are always iatrogenic. Serious complications are reported to occur infrequently, but in cases of the severe form of ovarian hyperstimulation syndrome (OHSS) secondary complications can lead to life-threatening conditions. Although the numerous publications on this subject give an impression of the frequency of occurrence of complications, hardly any reports exist about the incidence of the various complications in one programme. Berg and Lundkvist (20) reported on the clinical complications in 12 IVF clinics in Nordic countries. It is necessary to inform patients about the occurrence of complications in the very IVF programme they are about to join. In chapter 7 the incidence of the various clinical complications in the transport IVF programme at the Zuiderziekenhuis is reported, and the literature on this subject is reviewed.

One of the most controversial issues in IVF is the occurrence of triplets and higher-order pregnancies after replacement of three or more embryos. About 70% of triplet pregnancies occurring nowadays result from ovulation induction or ART (5, 6). The high costs (8) and the increased medical risks (7, 21) have been extensively described. The necessity of avoidance of triplets and higher-order pregnancies in IVF programmes has been stressed (8). Several authors suggested a limitation of the number of embryos to be replaced to achieve this (22, 23). Despite these recommendations the incidence of triplet pregnancies after IVF does not appear to decrease (24, 25), and replacement of even six or more embryos in patients with repeated previous IVF failures has been proposed (26). There are several explanations for this approach. In the first place, IVF programmes are mainly judged by the pregnancy rates obtained, and not by the frequency of proce-

dure-related complications. In some programmes this will lead to replacement of high numbers of embryos. Secondly, the increased morbidity and mortality of triplet and quadruplet pregnancies, and the psychological stress and social consequences for the parents might be underestimated, or seen as acceptable risks. In the third place, the availability of selective embryo reduction as a method to reduce high-order pregnancies to twins or even singletons, is probably seen by some as the solution for the occurrence of triplets and higher-order pregnancies. Chapter 8 is an analysis of the frequency of occurrence and of the obstetric outcome of triplet pregnancies in the transport IVF programme. This study was carried out to investigate whether triplet pregnancies should be completely prevented.

The expression of success rates in IVF

Before starting IVF treatment, couples need to be provided with adequate information about their chances to achieve a pregnancy. In the United Kingdom licensed IVF and donor insemination clinics therefore have a statutory duty to provide relevant information to patients before offering treatment (Human Fertilisation and Embryology Act 1990). In 1995 the "Patient's Guide to Donor Insemination and IVF Clinics" with the success rates of licensed IVF clinics was published (27). Using success rates for pre-treatment counselling should be done with care rates are not applicable to individuals and a possible reduction in pregnancy rate in successive cycles should be considered. This phenomenon has been described by several authors (28, 29), and is explained by heterogeneity of the population in terms of fecundity rate. Moreover, when cumulative pregnancy rates are used to express success, many programmes assume that patients who leave the programme after a failed attempt would have had the same probability of a pregnancy as those who continued. When patients drop out because of poor performance, e.g. few oocytes retrieved or failed fertilization, this assumption is of course incorrect. Consequently, the calculated cumulative pregnancy rate will be too high. In chapter 9 the cumulative pregnancy rate in the transport IVF programme is analyzed, and the validity of this rate is tested by determining whether there is a selective drop-out of patients with poor treatment prognosis.

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Part I

Logistical aspects of in vitro fertilization treatment

Chapter 1

Results of decentralized IVF treatment with transport and satellite clinics

Human Reproduction 1995;10:563-7.

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Summary

The results of in vitro fertilization (IVF) treatments, carried out in a university IVF centre, are compared with those obtained following 15-40 minutes transportation of oocytes from a transport IVF clinic to the central IVF laboratory of the university centre. Moreover, treatment results following monitoring of ovarian hyperstimulation in satellite clinics, combined with ovum retrieval at the transport clinic and transport of oocytes to the central IVF laboratory, are described.

In a total of 5540 IVF treatment cycles, 24-26 percent of viable pregnancies per embryo transfer were found in the three groups. Comparison of results, obtained with the three different treatment modalities, shows no negative influences of transporting oocytes from transport clinic to IVF laboratory and of monitoring ovarian hyperstimulation in satellite clinics. It is concluded that decentralization of the clinical phase of IVF treatment is possible. This leads to a more optimal use of existing laboratory facilities in large urban areas. It is stressed that efficient communication between satellite clinic, transport clinic and IVF laboratory is necessary for a decentralized IVF programme. To obtain good quality assurance, both the satellite clinic and the transport clinic must adhere to the same protocol.

Introduction

In The Netherlands the number of in vitro fertilization (IVF) laboratories has been limited to 12 by government regulation. Because the capacity of the IVF laboratory at IVF centres is usually larger than the treatment capacity of the clinical team, transport IVF, with carriage of aspirated oocytes from a transport IVF clinic to a central IVF laboratory, was introduced in 1984 (1-3). A transport IVF clinic is a facility where the complete clinical phase of the IVF treatment, including oocyte aspiration and follow-up after embryo transfer, can take place. The laboratory phase and embryo transfer are carried out at a different location. In a prospective pilot study, the results of transport IVF treatment cycles were compared with those in the central clinic (2). Transport of oocytes over 30-60 minutes proved to be possible without loss of fertilizability. The transport IVF programme at the Zuiderziekenhuis, Rotterdam, started in 1984. Satellite clinics, where monitoring of ovarian stimulation takes place according to the protocol of the transport clinic, were added to the programme in 1988 so as to limit travelling time and inconvenience for the patients.

A retrospective study with the objective of assessing the feasibility of large scale transport and satellite-transport IVF was carried out. The results of a 5 year period were analyzed and compared with concurrent results obtained in the central IVF unit at the University Hospital of Rotterdam. The results of cycles monitored at satellite clinics were compared with non-satellite monitored cycles. The results of the six different satellite clinics were compared to investigate if any variability could be found for the outcome of IVF treatment after monitoring of ovarian stimulation at these clinics.

Materials and methods

Three different treatment modalities were compared in this study. One group of patients (3333 cycles) recieved the clinical and the laboratory phases of IVF treatment at one location, the University Hospital of Rotterdam. A second group (2207 cycles) was treated at more than one location, recieving clinical treatment, including ovum retrieval, in the transport clinic, followed by carriage of the oocytes to the IVF laboratory at the University Hospital where the laboratory phase, including embryo transfer, took place. The transport clinic patient group was divided into two subgroups: 1637 cycles stimulated and monitored during ovarian stimulation at the transport clinic, and 570 cycles stimulated and monitored at one of the six satellite clinics participating in the programme. Patients travelled from the satellite clinics to the transport clinic for ovum retrieval. For these three treatment modalities the same IVF laboratory facilities were used.

Patient selection for the transport IVF programme was carried out at the Zuiderziekenhuis, the transport clinic. All patients were <43 years of age and were are accepted for the programme when they had a clear indication for IVF treatment and after serological tests (HBsAG, syphilis, human immunodeficiency virus) and routine physical examination. Ovarian stimulation consisted of human menopausal gonadotrophin (HMG) 150-450 IU i.m./day (Humegon®; Organon, Oss, The Netherlands), depending on age and IVF indication, starting on cycle day 3. Since the gradual introduction of gonadotropin-releasing hormone agonists, in the period 1989-1991, the short flare-up regime was used in most cases, starting on cycle day 1 until the day of human chorionic gonadotrophin (HCG) injection. Plasma estradiol measurements were not carried out in view of the results obtained at the central unit by Leerentveld et al. (4). Monitoring of follicle growth and endometrium development was performed by vaginal ultrasound measurements only. Oocyte retrieval was planned 35 hours after i.m. injection of 10,000 IU HCG (Pregnyl®; Organon) on the day the leading follicles reach a diameter of 18-20 mm, measured in three dimensions. Follicle aspiration was carried out by vaginal puncture under local anaesthesia. Oocyte isolation from the aspirates and subsequent laboratory procedures including embryo transfer were performed at the central IVF laboratory at the University Hospital of Rotterdam.

The distance between the transport clinic and the IVF laboratory was 7 km and the travelling time by car was 15-40 minutes. Oocytes were transported in their own follicular fluid in labelled vials placed in an insulated box with a preheated (35° C) block of aluminium. In this box the temperature remained constant (within 0.3° C) for at least one hour at an environmental temperature of 4° C. Regular tests of isothermic capacity were carried out. The box was carried to the central laboratory by the patient's partner. Semen was produced in the central laboratory. The laboratory procedure was recently described by Huisman et al. (5).

Luteal support was given with 1,500 IU HCG on the day of ovum retrieval (day 0) and on days 3, 6 and 9. When ≥15 follicles were retrieved, progesterone (200 mg vaginally twice daily; Progestan®; Organon) was prescribed for 15 days. In these cases HCG was withheld.

The number of embryos replaced (to a maximum of four) depended on the patient's age and the quality of the embryos. Patients <35 years of age were asked to consider replacement of not more than two embryos so as to prevent a triplet pregnancy. The final advice on the number of embryos to be replaced was given by the IVF laboratory staff.

A clinical pregnancy was defined by a positive urinary test 18 days after oocyte retrieval combined with the finding of a gestational sac two weeks later. A pregnancy was considered to be viable when fetal heart motion had been observed after seven weeks of amenorrhoea.

Satellite treatment was added to the transport IVF programme in 1988. Six clinics, at 100-200 km distance from Rotterdam, started to monitor ovarian stimulation for their own patients by

transvaginal ultrasound only. All decisions concerning patient selection, ovarian stimulation, monitoring protocol, timing of HCG injection and ovum retrieval were made according to the protocol of the transport clinic. Regular consultation with the transport clinic took place during ovarian stimulation. The satellite clinic contacted the transport clinic when follicle development allowed the timing of oocyte aspiration. A precise description of the number and size of follicles found during monitoring was given by the satellite physician. The day ovum retrieval was planned, the patient travelled to the transport clinic; oocyte aspiration with transportation of the oocytes to the central laboratory followed. In general, patients travelled to Rotterdam only three times: for intake and screening procedures, for follicle aspiration and for embryo transfer. During subsequent cycles only two visits were necessary.

A clinic was allowed to function as a satellite clinic after adequate training of the staff members involved in ovarian stimulation and monitoring. At two satellite clinics the staff members involved in IVF treatment previously participated in the IVF programme at the transport clinic for one year. Staff members at the other satellite clinics were offered 1 week of individual training at the transport clinic. Satellite physicians were provided with a detailed protocol for ovarian hyperstimulation and monitoring. An oocyte recovery rate of 70-80% of follicles reported to have a diameter $\geq$10 mm was considered acceptable. When this recovery rate was not achieved, the monitoring physician was contacted and the case discussed. The IVF results of each satellite clinic were evaluated on a regular basis and compared with the results of the transport clinic. Staff members at the transport clinic were expected to carry out a minimum number of fifty follicle aspirations a year for good quality assurance. No continuous record was kept of the oocyte retrieval rate, but regular random samples provided information on the individual physician's performance.

For statistical analysis, Student's t-test was used for the difference between two means and χ^2 test was used to compare the frequencies of observations. $P < 0.05$ was taken as the level of significance.

Results

The results of 2207 IVF treatments at the transport IVF clinic were compared with 3333 IVF treatments at the University Hospital of Rotterdam during the period January 1989 - December 1993. The two patient groups showed a significant difference in mean age (±SD): 33.1 ± 4.3 years and 34.0 ± 4.1 years respectively ($P < 0.01$). However, this difference of 0.9 years was small and had no clinical significance for the outcome of IVF treatment. The distribution of indications for

IVF treatment is shown in table 1. For the two main indications there appeared to be a significant difference in frequency. Tubal dysfunction was more often found in the University Hospital group ($P < 0.01$), while male subfertility was more frequent in the transport clinic group ($P < 0.01$).

The main results of IVF treatment at the two locations are shown in table 2. The mean numbers of oocytes and embryos per ovum retrieval differed significantly ($P < 0.01$) between transport clinic and University Hospital. The transport clinic had fewer embryo transfers ($P < 0.01$). A significantly higher number of embryos was transferred in the University Hospital group ($P < 0.01$), probably due to advice given more frequently to patients at the transport clinic to have only two embryos replaced. As a result, more cryopreservations ($P < 0.01$) per oocyte retrieval were seen in the transport clinic population. The percentages of clinical and viable pregnancies were similar in both groups. More multiple pregnancies ($P < 0.01$) were seen in the University Hospital group. The embryo implantation rates were similar in both groups.

Table 1. Indications for IVF in Transport Clinic and University Hospital

	Transport Clinic (%) (n = 2207)	University Hospital (%) (n = 3333)
Tubal dysfunction *	46	52
Endometriosis	7	4
Male subfertility *	23	16
Idiopathic	19	22
Rest and combinations	5	6

*Differences within rows significant ($P < 0.01$)

Starting in 1988, six satellite clinics monitored the ovarian stimulation for their own patients. As shown in table 3, the number of patients monitored at the satellite clinics increased since the satellite programme started.

The results of 1637 cycles monitored at the transport centre were compared with 570 cycles monitored at satellite clinics. A statistically significant difference was found between the two patient groups with regard to mean age (± SD): 33.3 ± 4.3 years and 32.6 ± 4.0 years respectively ($P < 0.01$). The indications for IVF in the two patient groups are shown in table 4. A statistically significant difference was found in the frequencies of tubal dysfunction ($P < 0.02$), and idiopathic infertility ($P < 0.01$).

The results of IVF treatment cycles monitored at the transport clinic, compared with the results of cycles monitored at the six satellite clinics, are shown in table 5. Statistically sigificant differences between transport and satellite clinics were found for the mean numbers of oocytes and embryos per retrieval ($P < 0.01$), and for the number of embryo transfers ($P < 0.01$). The mean number of embryos replaced was the same. The difference for cryopreservations was not significant. The percentages of clinical and viable pregnancies did not differ. The differences in percentage multiple pregnancies and in implantation rate were not significant. The incidence of severe ovarian hyperstimulation syndrome (OHSS), grade 5 or 6 according to the classification by Golan et al. (6), for the transport and satellite clinics population did not differ.

Table 2. Results of IVF Treatment in Transport Clinic and in University Hospital.

	Transport Clinic	University Hospital
Oocyte retrievals	2207	3333
Oocytes per retrieval (mean ± SD) *	9.7 ± 7.5	9.3± 6.5
Fertilization rate (%)	50.5	48.4
Embryos per retrieval (mean ± SD) *	4.9 ± 5.1	4.5± 4.7
Embryo transfers* (% †)	1786 (80.9)	2824 (84.8)
Embryos replaced (mean ± SD) *	2.6 ± 0.9	2.8± 1.0
Cryopreservations ‡ (% †)	642 (29.1)	845 (25.4)
Clinical pregnancies (% §)	512 (28.7)	810 (28.7)
Multiple pregnancies * (% ‖)	124 (24)	252 (31)
Twins (% ‖)	106 (20)	194 (24)
Triplets (% ‖)	18 (4)	56 (7)
Quadruplets (% ‖)		2 (0.2)
Embryo implantation rate (%)	14.0	14.1
Viable pregnancies (% §)	434 (24.3)	677 (24.0)

* Differences within rows significant ($P < 0.01$).
† Percentage per oocyte retrieval.
‡ Data on thawings and cryopregnancies are not included in this table.
§ Percentage per embryo transfer.
‖ Percentage per clinical pregnancy.

The results of IVF treatment obtained after monitoring in the six different satellite clinics are given in table 6. In the patient group at clinic 1, male subfertility (38%) was significantly more frequent as the indication for IVF ($P < 0.01$). This was associated with a significantly lower fer-

tilization rate ($P < 0.01$). Significantly less frequent male subfertility (13%) in the group at clinic 2 ($P < 0.02$) was associated with a higher fertilization rate ($P < 0.01$). A higher embryo implantation rate was found in the clinic 1 group ($P < 0.05$). The differences between the six clinics for percentages embryo transfers, clinical pregnancies and ongoing pregnancies were not statistically significant.

Table 3. Number of Transport IVF Cycles Monitored at Transport Clinic and Satellite Clinics.

Year	Total no.cycles	Transport Clinic	Satellite Clinics
1989	229	196 (86)	33 (14)
1990	363	305 (84)	58 (16)
1991	486	359 (74)	127 (26)
1992	555	391 (70)	164 (30)
1993	574	386 (67)	188 (33)

Values in parentheses are percentages.

Table 4. Indications for IVF in Transport Clinic and Satellite Clinics

	Transport Clinic (%)	Satellite Clinics (%)
	(n = 1637)	(n = 570)
Tubal dysfunction *	48	40
Endometriosis	7	7
Male subfertility	22	25
Idiopathic †	18	23
Rest and combinations	5	5

* Difference within row significant ($P < 0.02$).

† Difference within row significant ($P < 0.01$).

Discussion

The results show that IVF treatment in combination with transport of oocytes and monitoring in a distant satellite clinic yields a similar percentage of viable pregnancies as compared to a complete treatment in one centre. The results also confirm earlier studies (1, 2, 7, 8).

Table 5. Results of IVF Treatment in the Transport Clinic only and in Combination with Satellite Clinics.

	Transport Clinic	Satellite Clinics
Oocyte retrievals	1637	570
Oocytes per retrieval (mean ± SD) *	9.9 ± 7.6	9.3 ± 6.5
Fertilization rate (%)	51.5	48.4
Embryos per retrieval (mean ± SD) *	5.1 ± 5.2	4.5 ± 4.7
Embryo transfers* (% †)	1347 (82.3)	439 (77.0)
Embryos replaced (mean ± SD)	2.6 ± 0.9	2.6 ± 0.9
Cryopreservations‡ (% †)	483 (29.5)	159 (27.9)
Clinical pregnancies (% §)	386 (28.7)	126 (28.7)
Multiple pregnancies (% ‖)	85 (22)	39 (31.0)
Twins (% ‖)	73 (19)	33 (26)
Triplets(% ‖)	12 (3)	6 (5)
Embryo implantation rate (%)	13.6	15.1
Viable pregnancies (% §)	321 (23.8)	113 (25.7)
Severe OHSS ¶ (% †)	13 (0.8)	5 (0.9)

* Differences within rows significant ($P < 0.01$).
† Percentage per oöcyte retrieval.
‡ Data on thawings and cryopregnancies are not included in this table.
§ Percentage per embryo transfer.
‖ Percentage per clinical pregnancy.
¶ Classification of Golan et al (6).

The replacement of significantly fewer embryos did not affect the percentage of clinical pregnancies obtained in the transport clinic group. In this group a statistically significant lower multiple pregnancy rate was found. These findings confirm an earlier study by Staessen et al. (9).
The maximum duration of oocyte transport in this study was 40 minutes. In this period the oocytes did not lose their capacity to be fertilized and to develop into a viable embryo. It is unknown whether transport of longer duration will yield similar results. Adequate temperature regulation

during transport must be guaranteed. The use of a preheated (35°C) aluminium block in an insulated box has proved to be adequate in our programme. No power source is needed during transport. In this way possible technical problems during transport are avoided. During transportation the oocytes remain in their own follicular fluid. Apparently this fluid exerts no harmful effects on the oocyte. The fertilization rate reported has been influenced by the high frequency of male subfertility as indication for IVF treatment.

No negative effect on the outcome of IVF treatment is caused by the monitoring of ovarian stimulation by physicians in satellite clinics. The incidence of severe OHSS in the patient group stimulated and monitored at the satellite clinics is not increased and is comparable to incidences reported earlier (10, 11). The use of satellite clinics in an IVF programme has been described by others (7, 12-14). The introduction of satellite clinics made our transport IVF programme more accessible to patients in other parts of the country. Travelling time and inconvenience for the patients are limited since the monitoring of ovarian stimulation is carried out in their local hospital by their own gynaecologist. When satellite physicians monitor ovarian stimulation for their own patients, arrangements have to be made to obtain quality assurance. Satellite physicians should be familiar with the technique of vaginal ultrasound measurement of follicles and follow the treatment protocol of the transport clinic. The transport clinic, being responsible for the complete clinical phase of the IVF treatment including possible complications, should always be available to patients and satellite physicians for consultation, since difficulties may arise when the liability for mistakes during the IVF treatment has to be defined (15). The result of each ovarian stimulation should be communicated to the transport clinic before oocyte retrieval is planned. Satellite clinics should be informed about the laboratory results of their own patients, and the outcome of the IVF treatment should be reported to the transport clinic.

To obtain satisfactory IVF results the quality of the IVF laboratory is of significant importance. Once a laboratory has obtained a consistently good pregnancy rate, it is preferable to use the services of this laboratory instead of starting a new IVF laboratory. It seems that in large urban areas a decentralized IVF programme, with a central laboratory, is to be preferred above a programme with several small laboratories with less experienced personnel. Moreover, a decentralized programme is a more cost-effective approach to large scale IVF treatment.

Table 6. Results of In Vitro Fertilization Treatment after Monitoring in the 6 Satellite Clinics.

Satellite clinic	Clinic 1	Clinic 2	Clinic 3	Clinic 4	Clinic 5	Clinic 6
Oocyte retrievals	85	119	175	108	59	24
Oocytes per retrieval (mean ± SD)	9.9 ± 7.3	8.8 ± 5.3	9.2 ± 6.2	10.4 ± 7.9	8.6 ± 6.2	8.4 ± 5.8
Fertilization rate (%)	43*	55*	47	50	48	51
Embryos per retrieval (mean ± SD)	4.3 ± 4.9	4.8 ± 4.0	4.3 ± 4.4	5.2 ± 5.4	4.1 ± 4.9	4.3 ± 4.2
Embryo transfers (% §)	58 (68)	100 (84)	133 (76)	86 (80)	46 (78)	18 (75)
Embryos replaced (mean ± SD)	2.5 ± 1.0	2.6 ± 0.9	2.4 ± 1.0	2.7 ± 0.8	2.6 ± 0.9	2.7 ± 0.8
Cryopreservations ‖ (% §)	20 (24)	34 (29)	42 (24)	41 (38)	12 (20)	10 (42)
Clinical pregnancies (% ¶)	18 (31)	31 (31)	38 (29)	19 (22)	13 (28)	7 (39)
Multiple pregnancies (% **)	8 (44)	13 (42)	5 (13)	10 (52)	3 (23)	
Twins (% **)	5 (28)	12 (39)	5 (13)	8 (42)	3 (23)	
Triplets (% **)	3 (17)	1 (3)		2 (10)		
Embryo implantation rate (%)	20.5‡	17.2	13.4	13.4	13.6	14.3
Viable pregnancies (% ¶)	18 (31)	27 (27)	29 (22)	18 (21)	10 (22)	7 (39)
Percentage male subfertility	38*	13†	25	18	25	25

*†‡ Significantly different from other clinics ($P < 0.01$, $P < 0.02$ and $P < 0.05$ respectively).

§ Percentage per oocyte retrieval.

¶ Percentage per embryo transfer.

‖ Data on thawings and cryopregnancies are not included in this table.

** Percentage per clinical pregnancy.

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Chapter 2

Minimal monitoring of ovarian hyperstimulation: a useful simplification of the clinical phase of in vitro fertilization

Fertility and Sterility 1995;64:552-6.

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Summary

To investigate the feasibility of IVF treatment with minimal monitoring during ovarian hyperstimulation, a retrospective analysis and a prospective study with a real-time control group was carried out. In a transport IVF programme with satellite clinics 100 consecutive IVF cycles monitored at the transport clinic (TC), and 100 concurrent consecutive cycles monitored at satellite clinics (SC) were compared retrospectively for the number of ultrasound measurements (USM) carried out during monitoring, and for results of IVF treatment. Both TC and SC used the same stimulation/monitoring protocol. No patient selection took place. All cycles resulted in oocyte retrieval. After introduction of a minimal monitoring protocol at TC, a prospective study was started comparing 100 minimal monitoring cycles at TC with 100 concurrent conventional monitoring cycles at SC, all resulting in oocyte aspiration. Patients entered the retrospective or prospective study only once. In all cases the same laboratory facility was used. Monitoring of ovarian hyperstimulation was done with USM only.

Retrospective analysis showed no difference for the average number of USM at TC and SC; 2.8 ± 0.9 and 3.0 ± 1.0. No differences were found in the number of ongoing pregnancies obtained in the two groups; 22 and 18 respectively. One case of severe OHSS occurred in the SC group. Introduction of minimal monitoring at TC gave a significant reduction of the average number of USM at TC compared to SC where conventional monitoring continued to be used; 1.5 ± 0.8 vs. 2.8 ± 0.9. Ongoing pregnancies at TC and SC numbered 33 and 26 respectively. In both groups one patient developed severe OHSS. Sixty-two percent of cycles at TC were monitored with one USM only. No cancellations for impending OHSS occurred during the study period.

Conclusion: A large group of patients need only one USM during monitoring of ovarian hyperstimulation. Minimal monitoring gives a useful further simplification of the clinical phase of IVF treatment without adverse effects on treatment outcome and incidence of OHSS.

Introduction

In general ovarian hyperstimulation for in vitro fertilization (IVF) is carefully monitored in order to determine the appropriate moment of oocyte retrieval, and to screen for signs of impending overstimulation, possibly resulting in the development of the ovarian hyperstimulation syndrome (OHSS). In most IVF programmes monitoring is carried out by repeated ultrasound scans and plasma estradiol (E_2) measurements. Close monitoring is time-consuming, expensive and inconvenient for the patient. The necessity of close monitoring has been debated (1, 2, 3).

Succesful IVF cycles have appeared to be equally distributed over the entire range of plasma E_2 levels measured during ovarian stimulation. Therefore, the importance of E_2 measurements for daily treatment policy with respect to timing of human chorionic gonadotrophin (HCG) administration should be reconsidered (4). Some IVF programmes have abandoned the use of hormone assays completely (3, 5, 6). Further simplification of IVF treatment by reducing ovarian stimulation monitoring to a minimum, will cause less stress and inconvenience to the patient. In order to further minimize the monitoring procedure, a minimal monitoring protocol was evaluated in a prospective study.

Materials and methods

In a transport IVF programme with satellite clinics the transport clinic (Zuiderziekenhuis) collaborates with the IVF laboratory at the University Hospital of Rotterdam. The complete clinical phase of IVF treatment: patient selection, determination of stimulation schedule, monitoring of ovarian stimulation, ovum pickup and follow-up after embryo transfer, takes place at the transport clinic (TC). In the IVF laboratory at a separate location the laboratory phase including embryo transfer is carried out. Satellite clinics (SC) carry out stimulation and monitoring of ovarian hyperstimulation according to an identical protocol. All patients are treated with gonadotrophin-releasing hormone agonists (GnRH-a); in most cases the flare-up schedule is used. Ovarian stimulation consists of human menopausal gonadotrophin (HMG, Humegon®; Organon, Oss, The Netherlands) 150-450 IU/day, depending on age and indication for IVF, starting on cycle day 3

until the day of HCG injection. Monitoring of controlled ovarian hyperstimulation (COH) is done with transvaginal ultrasound measurements (USM) only. At the transport clinic eight members of staff are involved in monitoring; at the satellite clinics one to three. When more than 35 developing follicles are seen during monitoring, the cycle is cancelled by withholding HCG and continuing GnRH-a in order to reduce the chance of development of OHSS. Human chorionic gonadotrophin 10,000 IU (Pregnyl®; Organon) is given when > 50% of the leading follicles have reached an average diameter of 18-20 mm, followed by ovum pickup 35 hours later. Satellite clinics contact the transport clinic when follicle size allows planning of HCG injection and ovum pickup. Oocyte retrieval is done under local anaesthesia, transvaginally and ultrasound guided. After the ovum pickup the patient's partner carries the aspirates in an insulated box containing a preheated (35° C) aluminium block to the central IVF laboratory at the University Hospital, distance 7 km, travelling time 15-40 minutes.

Oocyte isolation from the aspirates and subsequent laboratory procedures including embryo transfer take place at the IVF laboratory. The laboratory procedure has been described by Huisman et al. (7). Luteal support is given with 1,500 IU HCG on the day of ovum pickup (day 0) and on days 3, 6 and 9. When 15 or more follicles are found during ovum pickup, progesterone 200 mg (Progestan®; Organon) vaginally, twice daily, is prescribed for 15 days. In these cases HCG is withheld. A clinical pregnancy is defined by a positive urinary test 18 days after oocyte retrieval combined with the finding of a gestational sac during ultrasound scanning two weeks later. A pregnancy is defined as ongoing when fetal heart motion is observed after 12 weeks of amenorrhoea.

With the conventional monitoring schedule, the first ultrasound measurement was carried out after 5 days of stimulation with HMG. Timing of subsequent USM depended on findings during first USM. Monitoring continued until the leading follicles reach an average diameter of 18-20 mm and HCG is given.

In order to compare the control data obtained in the two clinical settings, 100 consecutive IVF cycles, resulting in ovum pickup, monitored at the transport clinic and 100 consecutive cycles, resulting in ovum pickup, monitored at SC were compared with regard to the number of USM during COH and the outcome of IVF treatment. This control study was included to investigate whether the monitoring policies at TC and SC were similar, and whether results of IVF treatment differed. If not, the patient group monitored at SC could serve as a control group in a prospective study.

In the prospective study the monitoring schedule at TC was minimized. The first USM was carried out after 7 days of HMG, and, anticipating follicular growth of 1_-2 mm/day, HCG injection and ovum pickup were planned at that very moment, provided > 25% of the follicles had

reached an average diameter ≥ 14 mm. If these criteria were met, stimulation was continued, when necessary, for up to 4 days without any additonal USM and oocyte retrieval was planned. When findings during first USM did not meet the above mentioned criteria, a follow-up USM was performed. The concurrent patient group at the SC continued to be monitored according to the conventional protocol.

Patients entered the retrospective or prospective study only once. The OHSS classification of Golan et al.(8) was used in this study. Statistical analysis was done with χ^2 test or Student's t-test when appropriate.

Results

Retrospectively 100 consecutive IVF cycles monitored at TC were compared with 100 concurrent consecutive cycles monitored at SC. No patient selection took place. In both groups the same monitoring protocol was used. During the period in which these cycles were monitored no cancellation of IVF cycles for impending development of OHSS occurred. No statistically significant difference in age of the patients of TC and SC was found: 31.9 ± 4.6 and 32.6 ± 4.8 years (mean ± SD). The results are shown in table 1. No differences were found between the two groups for the mean duration of COH with HMG and the number of ultrasound measurements carried out during stimulation. The clinical pregnancy rates: 29% (confidence interval [C.I.] 20-39) versus 21% (C.I. 13-30), and the ongoing pregnancy rates: 22% (C.I. 14-31) and 18% (C.I. 11-27) did not differ. For the frequency of multiple pregnancies a statistically significant difference was found that can not be explained. One case of OHSS grade 4 occurred in the SC group.

The results of the prospective study with minimal monitoring of 100 consecutive IVF cycles at the TC and conventional monitoring of 100 concurrent consecutive cycles at the SC are shown in table 2. During the study period no cancellations for impending OHSS occurred. No patient selection took place. No difference was found for the mean age in the two groups: 32.2 ± 4.8, and 32.9 ± 4.3 years. The results of the prospective study are shown in table 2. Minimal monitoring during COH resulted in a significant reduction in the number of ultrasound measurements carried out. The results in table 2 indicate that the minimal monitoring protocol had no adverse effects on the outcome of IVF treatment. The duration of COH and the oocyte yield in the two groups did not differ. The differences in clinical pregnancy rates: 38% (C.I. 28-48) versus 28% (C.I. 19-38), and ongoing pregnancy rates: 33% (C.I. 24-43) versus 26% (C.I. 18-36), were not significant. In both groups one case of OHSS grade 4 occurred. The pregnancy rates in the prospectively studied

group were higher when compared to the retrospectively reviewed populations. However, the difference was not statistically significant ($P > 0.05$).

Table 1. Treatment Results of Retrospective Analysis with Conventional Monitoring at Transport and Satellite Clinics.

	Conventional monitoring Transport Clinic	Conventional monitoring Satellite Clinics
Ovum pickups	100	100
Flare-up schedule GnRH-a	85	88
Days hMG *	9.2 ± 2.2	9.1 ± 2.4
Number of USM *	2.8 ± 0.9	3.0 ± 1.0
Oocytes at pickup *	9.4 ± 6.8	9.2 ± 6.9
Embryos *	4.9 ± 5.1	5.5 ± 5.3
Cryopreservations †	38	40
Embryo transfers	76	82
Embryos replaced *‡	2.4 ± 0.5	2.5 ± 0.5
Clinical pregnancies	29	21
Multiple pregnancies §‖	3 (14)	10 (55)
Ongoing pregnancies	22	18
Embryo implantation rate	17.7 %	16.2 %
Severe OHSS ¶	0	1

* Values are means ± SD.

† Data on thawings and cryopregnancies are not included in this table.

‡ Per embryo transfer.

§Values in parentheses are percentages per clinical pregnancy.

‖ Significant difference TC versus SC, $P < 0.05$. Other comparisons not significantly different.

¶ Classification Golan (8)

The number of ultrasound measurements during COH and the duration of HMG stimulation in the prospective study is shown in figures 1 and 2. At TC, where the minimal monitoring protocol was used, 62% of oocyte retrievals could be planned after one ultrasound measurement only. The remaining patients needed a more individual approach. The new monitoring protocol resulted in an almost 50% reduction of patient visits to the hospital during the stimulation phase.

Table 2. Treatment Results of Prospective Study with Minimal Monitoring at Transport Clinic and Conventional Monitoring at Satellite Clinics.

	Minimal monitoring Transport Clinic	Conventional monitoring Satellite Clinics
Ovum pickups	100	100
Flare-up schedule GnRH-a	93	96
Days HMG *	9.6 ± 2.5	9.4 ± 2.5
Number of USM *†	1.5 ± 0.8	2.8 ± 0.9
Oocytes at pickup *	9.2 ± 6.8	8.7 ± 6.3
Embryos *	5.0 ± 4.2	5.1 ± 4.9
Cryopreservations ‡	36	34
Embryo transfers	92	87
Embryos replaced *§	2.2 ± 0.7	2.3 ± 0.7
Clinical pregnancies	38	28
Multiple pregnancies ‖	6 (16)	8 (29)
Ongoing pregnancies	33	26
Embryo implantation rate	22.1 %	18.2 %
Severe OHSS ¶	1	1

* Values are means ± SD.

† Significant difference, transport versus satellite clinics, $P < 0.0001$. All other comparisons not significantly different.

‡ Data on thawings and cryopreservations are not included in this table.

§ Per embryo transfer.

‖ Values in parentheses are percentages per clinical pregnancy.

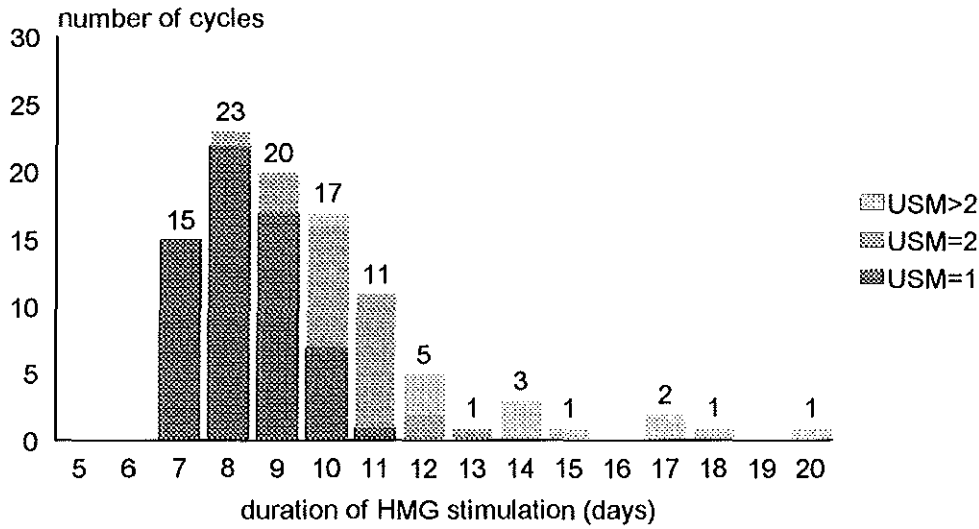
¶ Classification Golan (8)

Discussion

A major clinical development in IVF treatment during the past years has been the simplification of the treatment procedure (2). The laparoscopic oocyte retrieval has been replaced by the ultrasound guided transvaginal oocyte aspiration. Precise timing of HCG injection and ovum pickup 35 hours later have proven to be unnecessary after pituitary desensitization with GnRH-a (2, 9). The intensity of the monitoring of IVF cycles has already been reduced in several programmes (3). Monitoring during COH for IVF is carried out in order to determine the appropriate moment

of HCG injection and oocyte retrieval, and to screen for signs of impending development of OHSS. Repeated ultrasound scans and hormone assays are inconvenient, expensive and time-consuming. The necessity of intensive monitoring during ovarian hyperstimulation has been debated. Programmed IVF, using clomiphene with HMG on alternate days for stimulation, with limited monitoring only, has been recommended before (1, 5, 10).

Figure 1. The number of USM and duration of HMG stimulation with minimal monitoring at the Transport Clinic.

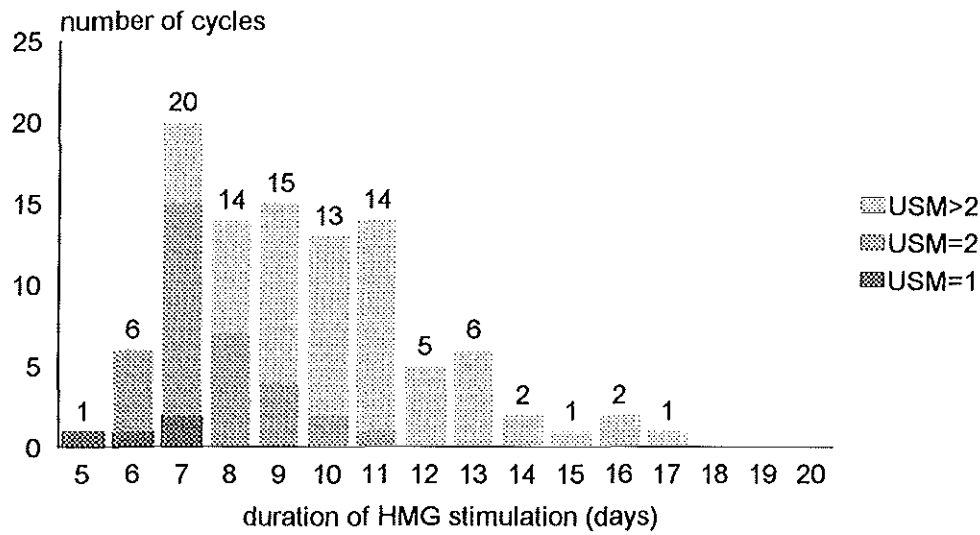


With the use of the GnRH-a, urine tests to screen for early luteinizing hormone peaks are not necessary anymore. High or low E_2 levels were found not to be detrimental to the clinical outcome of IVF treatment (4, 11). IVF pregnancies occur in the presence of a wide range of E_2 levels during the three day period preceding follicle aspiration, indicating that E_2 measurements are not necessary for succesful pregnancy induction (4). For these reasons the use of hormone assays has been abandoned completely in some programmes (3, 5, 6, 12). In these programmes ultrasonic scanning of follicular size is used as the sole index of follicular maturity. An average number of three ultrasound scans during each treatment cycle has been reported (3).

In our transport IVF programme, monitoring of COH has always been limited to ultrasound scans only. In order to reduce the monitoring procedure further, a minimal monitoring protocol was introduced. The first ultrasound scan was carried out after 7 days of HMG administration. When the criteria described were met, normal further development of follicles was anticipated and

oocyte retrieval was planned. After introduction of this monitoring protocol the number of ultrasound scans and patient visits during the stimulation phase was reduced by almost 50%. Sixty-two percent of IVF cycles were monitored with one ultrasound measurement only. No adverse effects on the outcome of IVF treatment were found. Although the pregnancy rates in the group with minimal monitoring were higher compared to the group with conventional monitoring, the difference was not statistically significant. Finding a difference for pregnancy rate between the two groups was not an objective of this study and would have called for a far larger sample size. In addition, a better outcome of IVF treatment is not to be expected from less intensive monitoring. Pregnancy rates in the prospectively studied groups were higher compared to the retrospectively studied groups, although the difference was not statistically significant. In an earlier study evaluating the results of 2207 consecutive IVF cycles in the same programme, a pregnancy rate of 28.7% was found for both transport clinic and satellite clinics (13). It appears that the results of the studies described illustrate the occasionally occurring fluctuations of results in an IVF programme.

Figure 2. The number of USM and duration of HMG stimulation with conventional monitoring at Satellite Clinics.



In IVF treatment the development of mild ovarian hyperstimulation, allowing recruitment of a cohort of mature follicles, is a primary goal. The development of an OHSS however, can not be prevented in all IVF cycles. Estradiol peak level and rate of increase, dose of HMG, size and

number of follicles developing and the number of oocytes collected are parameters used for the prediction of the development of OHSS. A considerable overlap of distributions of these values between control and OHSS populations has been found (14). In order to increase predictability of OHSS, a formula for pre-oocyte retrieval conditions was established in a multiple discriminant analysis, yielding a prediction rate for the development of moderate or severe OHSS of 76.1% with a false negative rate of 18.1% (15). This formula can only be used after frequent hormone assays have been carried out and calls for an intensive and expensive monitoring procedure. However, OHSS is not prevented in all cases. The value of ultrasonography in predicting the development of OHSS by number and size of follicles has been assessed (16). In view of this, cycles in the present study were only cancelled for impending development of OHSS when more than 35 follicles, with a diameter $\geq$ 10 mm, were seen to develop during COH. No cancellations for this reason occurred during the study period. Nevertheless, three cases of severe OHSS were found in our study groups, one in the group with minimal monitoring, two in the groups with conventional monitoring. This incidence of OHSS (0.75%) is comparable with the results of programmes using combined ultrasound measurements and hormone assays (17).

In conclusion: minimal monitoring simplifies IVF treatment, causes less inconvenience for the patient and is more cost-effective. The outcome of IVF treatment is not affected and the incidence of development of OHSS does not appear to be increased.

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Chapter 3

The logistical advantages of gonadotrophin-releasing hormone agonists in IVF

(Submitted for publication)

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Summary

For routine use of gonadotrophin-releasing hormone agonists (GnRH-a) in an IVF programme the short flare-up protocol and the long protocol give comparable clinical results. Both protocols are useful for organizational purposes and offer important logistical advantages for IVF programmes. In terms of cost, time and inconvenience for patients the short flare-up protocol seems to be the most appropriate regimen for routine use. The intranasal administration of GnRH-a appears to be equally efficacious when compared with the subcutaneous route.

Introduction

Gonadotrophin-releasing hormone agonists combined with human menopausal gonadotrophin (HMG) are now widely used for controlled ovarian hyperstimulation (COH) in IVF programmes. Initially GnRH-a were introduced as a pretreatment before COH after failed IVF attempts (1-5). The administration of GnRH-a gives an initial stimulatory phase ("flare-up") followed by down-regulation of pituitary GnRH receptors, resulting in the reduction of the release of gonadotrophins by the pituitary gland. This suppressive effect of agonists on the endogenous gonadotrophins leads to ovarian quiescence, and prevents the recruitment of follicles induced by pituitary FSH, that is optimally initiated in the late luteal phase of the preceding cycle. The administration of gonadotrophins when pituitary suppression and ovarian inactivity are achieved, results in a synchronous development of a larger cohort of follicles, and consequently in a larger oocyte yield

and lower cancellation rate (6). With the administration of GnRH-a during COH, the developing oocytes are protected from perturberations in luteinizing hormone (LH) release which are thought to be detrimental to the normal maturation of the egg (7, 8). Also, beneficial effects in patients with high tonic LH levels have been described (9). Smitz et al. (3) reported occurrence of a premature surge of LH without the use of agonists in 15% to 30% of patients. Loumaye (10) found that without the use of GnRH-a one half of oocyte collections were timed by an endogenous LH surge, whereas the other half were timed by HCG administration. Through the control of the preovulatory LH surge spontaneous ovulations are prevented and expensive and cumbersome monitoring in order to detect a putative LH surge is not necessary. Therefore, the timing of oocyte retrieval has become less critical (11), giving considerable organizational advantages for IVF programmes, and making programmed IVF possible (12, 13).

Different protocols have been developed for the use of GnRH-a in IVF: the long down-regulating protocol, and the short 'flare-up' and ultra-short protocol. Some aspects of these protocols will be discussed with an emphasis on the logistical advantages of the use of agonists during COH. Furthermore, the experiences with GnRH-a in the transport IVF programme at the Zuiderziekenhuis, Rotterdam, are reported.

The long down-regulating protocol

The logistic advantages of the use of the long down-regulating protocol in IVF have been stressed by several authors (14-16). Wikland et al. (15) described a considerable simplification of the monitoring of COH, and avoidance of oocyte collections during weekends, without a decrease in pregnancy rate. In the long down-regulating protocol the administration of gonadotrophins for ovarian hyperstimulation is started after pituitary down-regulation is achieved.

Conflicting reports have been published with respect to the preferred phase in the menstrual cycle for starting desensitization. Good results with the initiation of GnRH-a in the early follicular phase have been reported by Pellicer et al. (17). Urbancsek and Witthaus (18) conducted a multicenter study and found that clinical and live birth rates are higher when the agonist was started in the midluteal phase rather than in the early follicular phase. Two recent studies found comparable IVF results after starting down-regulation in different phases in the menstrual cycle: Ferraretti et al. (19) found that agonists can be started irrespective of the cycle phase, and Konaveeti et al. (16) reported that both follicular and luteal phase initiation of GnRH-a are equally efficacious. Although long-acting GnRH-a is more convenient for patients than a daily administration of a short-acting one, it has been reported that long-acting GnRH-a might interfere with the luteal

phase and embryo development (20, 21). Therefore, short-acting GnRH-a should be preferred for COH.

A disadvantage of starting the administration of agonists in the luteal phase is the possible presence of a pregnancy. There are indications that GnRH-a may have a detrimental effect on corpus luteum function by a direct effect on the ovary (22). In experimental studies an increase in major fetal abnormalities has been found after administration of a depot suspension of GnRH-a on day 6 of pregnancy in rabbits (23). Although several authors reported normal pregnancies despite the use of GnRH-a in the first trimester of pregnancy (24-28), the potential for teratogenicity may exist and caution is indicated.

With the use of GnRH-a in the long protocol the costs of medication in IVF have increased. The treatment cycle is lengthened by the time needed to achieve down-regulation. Complete down-regulation can be assessed by serum estradiol (E_2) estimations and ultrasound examinations of the ovaries to screen for follicular activity. Therefore, although the long protocol has practical advantages for the organization of IVF programmes, the disadvantages for patients in terms of cost, time, and inconvenience can not be ignored.

The short flare-up protocol

The short flare-up protocol makes use of the initial release of endogenous gonadotrophins to augment follicular recruitment and the suppression of endogenous gonadotrophin secretion thereafter. In this protocol the agonist is started in the early follicular phase of the cycle, followed by the administration of gonadotrophins 1-3 days later. When the agonist is continued until the day of HCG administration, the protocol is called the short protocol, and, if it is administered for an initial 3 days only the ultra-short protocol. Initially meant to block the LH surge during COH (29), it appeared that the "flare-up" effect, i.e., the initial gonadotrophic stimulation induced by the analogue prior to gonadotrophic desensitization, could play a role in the shorter duration of ovarian stimulation (30, 31). It has been reported that the flare-up protocol might be the therapy of choice for patients at risk of a poor ovarian response during COH (32, 33).

The short-term GnRH-a regimen can be combined with progestagen pretreatment to allow for an advanced scheduling of the moment of oocyte retrieval (34). It has been shown that the ovarian response to COH with the flare-up protocol is not affected by progestagen administration in the previous cycle, irrespective of the patient's age (35, 36). Although pretreatment with norethisterone decreases the E_2 flare-up, the predictive value of the initial change in E_2 level reported by Winslow et al. (37) is not changed, and the outcome of the IVF cycle is not influenced (38).

Between January 1989 and August 1996, 1825 patients had their first IVF cycle in the transport IVF programme at the Zuiderziekenhuis in Rotterdam. The flare-up protocol was used in 1598 cases (88%), the long protocol with a midluteal start of GnRH-a in 89 cases (5%). The remaining 138 patients (7%) were treated without GnRH-a during COH in their first IVF cycle. Monitoring of COH was carried out without E_2 determinations and with transvaginal ultrasound measurements only. Despite the limited monitoring procedure (39), a spontaneous ovulation occurred in only 5 cases (0.3%) in the group treated with the flare-up protocol. This compared favourably with the 4 cases (3%) of spontaneous ovulation in the patient group treated without agonists ($P < 0.01$, χ^2 test). Spontaneous ovulation did not occur in the group of 89 patients treated with the long protocol. In the flare-up protocol four different GnRH agonists were used, three for subcutaneous injection: leuprolide (Lucrin®, Abbott), buserelin (Suprefact®, Hoechst), and triptorelin (Decapeptyl®, Ferring), and one for intranasal use: nafarelin (Synarel®, Searle).

Table 1. Results of First IVF Cycles with the Flare-up Protocol.

	Leuprolide 1 mg s.c.	Buserelin 500 µg s.c.	Triptorelin 0.1 mg s.c.	Nafarelin intranasal 200 µg b.d.
Started cycles	720	114	387	377
Cancelled, poor response *	20 (2.8)	4 (3.5)	7 (1.8)	8 (2.1)
Cancelled, other reasons	2	0	1	2
Premature ovulation	1	1	2	1
Oocyte retrievals	697	109	377	366
Oocytes retrieved †	10.0 ± 6.8	12.3 ± 10.3	11.1 ± 7.6	10.4 ± 7.0
Clinical pregnancies ‡	168 (24)	21 (19)	82 (22)	92 (25)
Ongoing pregnancies ‡	144 (21)	18 (17)	63 (17)	76 (21)

* Values in parentheses are percentages of started cycles.

‡ Values in parentheses are percentages per oocyte retrieval.

† Means ± SD.

The results of these cycles are shown in table 1. Obviously, the clinical results of the four agonists in the short protocol are comparable. In table 2 the results of the two different routes of administration of GnRH-a, intranasally for nafarelin and subcutaneously for buserelin, leuprolide and triptorelin, are compared. No statistically significant differences were found for the different parameters (χ^2 test and Student's t-test used where appropriate). In this retrospective analysis of our

population, the intranasal administration of GnRH-a appeared to be equally efficacious when compared with the subcutaneous administration.

The easy intranasal administration of nafarelin and the good clinical results described in several studies (40, 41) have made nafarelin an option for use in COH for IVF. The efficacy of intranasal nafarelin in the flare-up protocol has been described (42). The results obtained with nafarelin in the short protocol in the transport IVF programme confirm these findings.

Table 2. Results of the Flare-up Protocol with Intranasal versus Subcutaneous GnRH-a in First IVF Cycles.

	GnRH-a s.c.	GnRH-a intranasal	*P* value
Started cycles	1221	375	
Cancelled poor response *	31 (2.5)	8 (2.1)	NS†
Premature ovulation *	4 (0.3)	1 (0.3)	NS
Oocyte retrievals	1183	366	
Oocytes retrieved ‡	10.6 ± 7.5	10.4 ± 7.0	NS
Clinical pregnancies §	271 (23)	92 (25)	NS
Ongoing pregnancies §	225 (19)	76 (21)	NS

* Values in parentheses are percentages of started cycles.

† NS, not significant, χ^2 test.

‡ Means ± SD.

§ Values in parentheses are percentages per oocyte retrieval.

Long protocol versus short protocol

Although several studies have shown that the combination of GnRH-a with HMG results in higher pregnancy and live birth rates and reduced cancellations due to poor follicular response or premature luteinizing hormone surges (6, 43, 44), the routine use of GnRH-a for patients undergoing IVF appears to have more practical than medical advantages (45, 46, 19). However, the combination of GnRH-a and gonadotropins has become the most widely used regimen for COH in IVF programmes. The different protocols used for GnRH-a have been compared in several studies. In the first randomized study comparing long and short protocols Frydman et al. (47) concluded that the clinical results of the two protocols are similar, and that other factors should be taken into account when deciding what protocol is to be used. Conflicting results have been published by other

authors. In a meta-analysis of ten prospective randomized clinical trials, Hughes et al. (44) found no significant differences between the long and short protocol with respect to cycle cancellation and pregnancy rates. Tan et al. (48) reported higher probabilities of conception and live birth with the long protocol, but this study was retrospective.

In the short protocol the initial release of endogenous gonadotrophins augments follicular recruitment. A shorter duration of stimulation and lower doses of exogenous gonadotrophins needed using the short protocol have been reported in comparative studies (34, 49). However, in several other studies no effect of the "flare-up" effect itself could be demonstrated when the stimulation requirements in the short and the long protocol were compared (47, 49-51). In patients at risk of a poor response the short flare-up regimen seems to have advantages over the long protocol (32, 33). Muasher (32) stated that prior suppression with GnRH-a in poor responders might result in excessive dampening of the ovarian response. The suppression of LH levels throughout the late follicular phase may not be as complete with the short protocol as with the long protocol. Elevated endogenous LH levels have been reported to lead to a higher incidence of poor fertilization, failure of implantation and early abortion (7, 8). Acharya et al (50) found no premature LH surge with the flare-up protocol in any of the cycles studied. However, LH surges might occur more frequently with the ultra-short protocol (52).

The logistical advantages of the long and the short protocol are comparable as programmed IVF has been described using both regimens. The feasibility of minimal monitoring during COH with both protocols has also been reported (15, 39)

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Part II

Clinical aspects of in vitro fertilization

1.
Aspects of patient selection

Chapter 4

Age and IVF treatment

Assisted Reproduction Reviews 1995; 5: 66-73.

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Introduction

Delayed childbearing is increasingly common in the western world. The availability of measures for birth control, educational and career priorities for women, and the increased rates of divorce and remarriage are factors causing this phenomenon. Postponement of childbearing simultaneously increases the chance of exposure to sexually transmitted diseases and correlated infertility due to tubal dysfunction. Possible sub- or infertility will become evident at a higher age. The decrease in fecundity (ability to procreate) of women of advanced age is generally known. Schwartz and Mayaux (1) analysed the results of artificial insemination in 2193 nulliparous women with azoospermic husbands and found evidence for a decrease in fecundity of women after the age of 30. Postponement of childbearing resulting in prolonged exposure to factors affecting fecundity, combined with the natural reduction of fecundity, causes an increased proportion of patients in the higher age groups to consult infertility services. The patient's age should be taken into account when treatment choices are made to correct infertility.

Treatment of infertile patients, after documenting tubal patency, with ovarian stimulation and intra-uterine insemination has shown a very low success rate in women above 40 years of age. Frederick et al. (2) reported a 5% pregnancy rate per cycle in patients above 40 years compared to 10% in the group below 40 years. In these patients the most efficacious treatment for infertility should be chosen since they have only few reproductive years left. Consequently, assisted reproductive technologies (ART) are frequently applied in higher age groups. However, the limited results obtained in patients above the age of 40 years make the utilization of techniques for assisted reproduction controversial in these cases. Advanced age is correlated with a poor response to controlled ovarian hyperstimulation (COH) for IVF (3) and high cancellation rates during COH (4, 5). The pregnancy rates of patients above 40 years are decreased and the rates of miscarriage

are highly increased when compared to lower age groups. Padilla and Garcia (6) found a 9% ongoing pregnancy rate per embryo transfer (ET) in patients aged 37 years compared with a 26% ongoing pregnancy rate in patients younger than 30 years. Results from The American Fertility Society Registry (5) show a 10.8% delivery rate per ET in patients above 39 years compared to 30.4% below 40 years. Tan et al. (7) found a cumulative conception and livebirth rate after five treatment cycles of about 54% and 45% respectively at 20-34 years, compared with 20.2% and 14.4% above 40 years. Results of 492 IVF units, collected from national surveys and registries of different countries, over the period 1985-1989 show a live birth rate of 5.8% per transfer cycle above the age of 40 years (8). Check et al. (9) found a significant effect of age in patients above 35 years with multiple factors causing infertility in a prospective study.

The miscarriage rate in IVF pregnancies above 40 years varies between 41-62.5% of clinical pregnancies (5, 6, 8, 10). Poor results obtained in the patient group aged 40 years or more has led to an age limit for acceptance for IVF treatment in most government supported programmes. However, one can ask the question whether a strict age limit for acceptance of patients is justified since the patient's performance in ART appears to be more closely related to ovarian age than to chronological age (3). Other clinical parameters like prevalence of spontaneous menstrual cycles or even normal ovulatory cycles, can not be used for an adequate prediction of the response to COH (11). Endocrine parameters proved to be more useful as a prestimulation test and can be used for counselling patients on their chances for a succesful ovarian stimulation for IVF treatment. The determination of basal follicle-stimulating hormone (FSH) levels (3), clomiphene citrate stimulated FSH levels (12), and the gonadotrophin-releasing hormone agonist (GnRH-a) stimulation test (13) prior to COH have been proposed in this respect.

Several stimulation protocols have been developed in order to improve the outcome of IVF treatment in patients with a poor prognosis or a previous poor ovarian response to COH (14-18). More effective stimulation protocols might improve the IVF results in patients of the higher age groups.

Assisted hatching has been proposed as a method to improve the implantation rate in cases with a poor prognosis. Cohen (19) and Schoolcraft (20) advised this micromanipulative procedure for patients aged above 38 and 39 years respectively.

According to several authors replacement of more than two embryos should be avoided in IVF-cases with a good prognosis (21, 22). The embryo implantation rate has been reported to decline after the age of 35 and appears to be low in patients above 40 years (23, 24). In IVF programmes with similar results, replacement of 3 or 4 embryos seems justified after the age of 40 years, thereby enhancing chances for a favourable outcome. However, the prevalence of triplet pregnancies should remain a concern when decisions on embryo replacement policy are made.

Oocyte donation (OD), with oocytes obtained from a younger donor, appears to be a good alternative for women in the higher age groups who have failed to achieve a pregnancy with their own oocytes due to a poor response to ovarian stimulation (25-27). As a result of OD programmes, pregnancies and deliveries in elderly and postmenopausal women have been reported. Age limitation in OD programmes is highly controversial. By only selecting women in good health as acceptors of oocytes it is reasonable to expect that the incidence of complications will be reduced (28).

Methods to select patients in the higher age groups with a fair chance for a succesful outcome of IVF treatment, and the various possibilities to enhance the chances for a succesful pregnancy are discussed hereafter.

Prestimulation tests

Increased ovarian age, resulting in decreased ovarian function and diminished ovarian reserve, can cause a poor follicular response to COH (3). When a "poor ovarian response" occurs, meaning that only one or two follicles develop, the IVF cycle can be cancelled, or the procedure can be continued, consequently with a very limited chance of succes. It would be useful if patients with a poor response to ovarian stimulation could be identified before a time-consuming, stressfull and expensive IVF procedure has been started.

Muasher et al. (29) investigated the predictive value of basal gonadotrophins for the quality of COH in a prospective study. It was concluded that measurement of basal serum gonadotrophin levels offers the possibility to distinguish between populations of IVF patients who tend to behave differently in estradiol (E_2) response, numbers of oocytes obtained, embryos transferred and pregnancy rates. Follicle-stimulating hormone levels on cycle day 3 have also been shown to be predictive of IVF outcome by Scott et al. (30). The authors distinguished three patient groups with different basal FSH levels (< 15 mIU/ml, 15-24.9 mIU/ml and >25 mIU/ml) showing significantly different ongoing pregnancy rates (17.0%, 9.3% and 3.6%). Toner et al. (3) reported results of 1478 consecutive IVF cycles, analyzed to determine if basal FSH levels and age are independent predictors of IVF performance. Pregnancy rates declined as age and basal FSH increased. Pregnancy rates per attempt steadily declined as basal FSH increased up to 20 mIU/ml, thereafter the decline was abrupt. Less than 5% term pregnancies per IVF attempt were found in cases with a basal FSH > 25 mIU/ml. The relationship between increasing age and pregnancy rate per IVF attempt was steady throughout the observed age range. Ongoing pregnancy rates fell from 28% percent in patients in their mid-twenties to 12 % around the age of 40 years. The risk of cancella-

tion of the IVF attempt was strongly related to basal FSH level (FSH ≥ 30 mIU/ml : 40% cancellation rate). However, no relationship was found between cancellation rate and age. The authors conclude that the basal FSH level is a better predictor of IVF performance than age. The clomiphene citrate (CC) challenge test was used by Navot et al. (31) to prospectively assess the fertility potential in 51 women aged 35 or more with unexplained infertility. Although all women had a normal baseline FSH and regular menstrual periods, 18 had a high FSH response of 26 mIU/ml or more (> 2 standard deviations above control levels) after administration of 100 mg clomiphene from cycle day 5-9. In this group one out of 18 patients concieved compared to 14 of the 33 patients with a normal FSH response. There was no difference in age between the groups (40.0 and 39.6 years respectively).

Tanbo et al. (32) found poor responders to ovarian hyperstimulation with an abnormal FSH response to CC to have a low chance of success in further IVF attempts. The total pregnancy rate per patient, including spontaneous conceptions during the study period was 4% in the abnormal response group, compared to 33% in the normal response group. In a later prospective study, Tanbo et al. (12) showed the CC challenge test to have a better prospective value than the basal FSH level. In a group of 37 patients with an excessive FSH response to CC administration, 46 (85%) of 54 cycles were cancelled because of poor response to ovarian stimulation. Loumaye et al. (33) used the sum of basal FSH (FSH1) and FSH (FSH2) after CC intake as the parameter correlating best with a subsequent response to COH. This way the dependency on putative FSH assay inaccuracy and fluctuation of the FSH concentrations due to pulsatile excretion was reduced. The reference value for FSH1 + FSH2 was determined to be 26.03 mIU/ml. The number of follicles aspirated, oocytes retrieved and embryos obtained were on average six times lower in the 20 patients with a CC challenge test above this reference than in 20 controls with a test result within the reference limits. In the group with an abnormal test result no pregnancy was obtained.

Olivennes et al. (16) described the exogenous FSH ovarian reserve test. When a poor ovarian response is expected i.e. age > 37 years, or when cycle irregularities, ovarian pathology or poor response in previous cycles have been found, patients recieve 300 IU of purified FSH on the third day of the menstrual cycle. Basal FSH and E_2 level before FSH administration, and the E_2 level 24 hours later are determined. With a basal FSH > 10 mIU/ml and an E_2 increase < 30 pg/ml the prognosis was considered poor.

Padilla et al. (34) investigated the prognostic value of the early serum E_2 pattern in response to leuprolide acetate in a GnRH-a flare-up protocol. In patients with an absent early E_2 response before ovarian stimulation started on cycle day 5, the clinical pregnancy rate per cycle was 6% compared to 37% in the group with rising estradiol levels.

Winslow et al. (13) also described a GnRH-a stimulation test used in a group of 228 patients with a mean age of 36.6 ± 3.6 years. In contrast to Padilla et al. these authors found no significant correlation between the early E_2 pattern and the pregnancy rate. In their study they concluded that the initial change in E_2 level is a sensitive predictor of performance in the flare-up IVF cycle. Patients who had less than a doubling of their E_2 level on cycle day 3 compared to day 2 had a high cancellation rate (39%) during subsequent ovarian stimulation with gonadotrophins.

Controlled ovarian hyperstimulation in "poor responders"

Although the first successful IVF treatments were achieved in spontaneous cycles (35), COH is now used prior to oocyt retrieval. With COH, more oocytes can be obtained during follicle aspiration and a higher number of embryos available for replacement consequently leads to higher pregnancy rates (23). A poor response to ovarian stimulation is more frequently seen in higher age groups and this often leads to cancellation of IVF cycles. The definition of poor response differs between authors (15, 18, 24, 36). An E_2 level < 300 pg/mL and fewer than three follicles with diameter > 15 mm prior to administration of human chorionic gonadotrophin are the most frequently mentioned criteria. Studies with poor responders are characterized by a selection bias and results from different studies are frequently contradictive. Thus, proposing an optimal treatment for poor responders to COH is one of the challenges in assisted reproduction. Increase of the doses of human menopausal gonadotrophin (HMG) does not necessarily lead to a better follicular response. Interestingly, Ben-Rafael et al. (37) found a higher number of oocytes obtained after stimulation with 150 IU HMG daily as compared to that with 225 IU. Benadiva et al. (38) studied hormonal profiles and follicular growth in high and low responders with different doses of HMG. In low responders they found no positive effect of higher doses HMG on E_2 concentrations or on follicular development. However, the number of patients in this study was low (8 high responders versus 6 low responders) and the difference in mean age between the groups was considerable (31 ± 1.5 and 35 ± 1.8) although not significant in this small group. In support of this study, Karande et al. (39) found no improvement in oocyte yield after high-dose FSH stimulation at the onset of the menstrual cycle in low-responding patients. In the same study no beneficial effect was found of the administration of 6 ampules of pure FSH instead of 4. However, other authors have found a positive effect of the administration of higher doses of gonadotrophins in poor responders. Hoffmann et al. (18) treated patients with one or more previous poor responses to COH with high doses of FSH (450 IU instead of 300 IU daily). There was no difference in age (36.1 ± 3.3 and 36.3 ± 2.9 years respectively) or basal FSH levels between the two patient groups. High dose cy-

cles showed a significantly lower cancellation rate and a higher pregnancy rate. The numbers of oocytes retrieved and embryos transferred were also higher after administration of FSH 450 IU daily but these differences were not significant.

Doubling the HMG (225 IU to 450 IU) dose in the course of an IVF treatment cycle in low responders appeared not to be effective to enhance the ovarian response in a prospective, randomized study by Van Hooff et al. (40).

Changing a stimulation protocol using HMG only, to a protocol combining clomiphene citrate and HMG, does not improve the ovarian response in a group of poor responders as reported by Pellicer et al. (41). The authors observed no significant difference between the two stimulation protocols with regard to the cancellation rate or the number of oocytes retrieved per cycle.

The use of GnRH-a suppression followed by COH has generally proven to be useful in lowering cancellation rates, improving follicular growth and the yield of oocytes in IVF treatment(42, 43). Serafini (14) reported a beneficial effect of the use of GnRH-a in a group of poor responders with normal basal FSH levels. By contrast, other authors (15, 44) advise to avoid GnRH-a suppression prior to COH in low responders when the disorder is located at the ovarian level and not at the hypothalamic/pituitary level. The short flare-up GnRHa protocol seems however to have a place in the treatment of poor responders. Muasher (15) described a modified flare-up GnRHa protocol combined with high-dosage FSH (450-600 IU) in a step-down manner for previous poor responders. They reported a cancellation rate of 5% and a 21.3% clinical pregnancy rate per cycle.

Adjuvant growth hormone (GH) treatment, after pituitary suppression, has been proposed for ovarian stimulation in order to improve the results. Owen et al. (45) investigated the effect of GH cotreatment in 25 IVF patients who had previously responded suboptimally to ovarian stimulation. In their study the authors found that a significantly higher number of follicles developed during stimulation with GH cotreatment. However, from this study it can not be concluded that GH is beneficial to poor responders in general. A previous suboptimal result was defined as less than 6 oocytes obtained during a previous IVF cycle. Besides, the augmentation of ovarian response the authors found, was only seen in the large subgroup with polycystic ovarian syndrome. Hughes et al. (46) and Bergh et al. (47) could not find a beneficial effect of the use of GH in randomized, double-blind, placebo-controlled studies. Therefore, it can be concluded that the use of GH in patients with a previous poor response to COH due to increased ovarian age is not indicated.

Use of assisted hatching

Impairment of healthy embryos to hatch from the zona pellucida has been postulated by Cohen et al. (48) as one of the causes for early embryonic demise in IVF procedures. A micromanipulation to promote hatching by introducing an artificial incision in the zona pellucida of early cleaved embryos prior to replacement was described. According to the authors this procedure facilitates the hatching process mechanically and so they called it assisted hatching. One should bear in mind however, that the occurrence of hatching in vivo has not been demonstrated in the human species. In addition, in the mouse, where blastocysts hatch in vitro, hatching has never been observed in vivo during a normal pregnancy. The authors report a significant difference in embryo implantation rate (EIR) of 23% after assisted hatching compared with 11% in the control group. In a later publication Cohen (19) reported three trials designed to investigate the effect of selective assisted hatching by drilling of the zona pellucida of embryos. Patients whose embryos had thick zonae ($\geq$ 15 mm) derived a benefit from zona drilling, while embryos with thin zonae ($<$ 13 mm) may be jeopardized by the procedure. Although more embryos appeared viable in all age groups, statistical significance could only be demonstrated in patients above 38 years of age. In this group the EIR improved from 3% to 16%. Another report on the improved IVF results after assisted hatching in poor prognosis patients confirmed these findings. Schoolcraft et al. (20) defined poor prognosis as: basal FSH $>$ 10 mIU/ml, age $\geq$ 39 years or multiple prior IVF failures. In a controlled study 122 embryos underwent assisted hatching. The implantation rate of these embryos was high: 33% versus 6% for the 185 non-treated embryos in the control group. This difference was highly significant. The incidence of ongoing pregnancy in the assisted hatching group was 64% compared to 19% in the control group. Earlier implantation after assisted hatching was reported by Liu et al. (49). The implantation time, calculated by assessing human chorionic gonadotrophin serum levels, proved to be significantly shorter in the assisted hatching group. The authors postulated that assisted hatching not only enhances embryo implantation by mechanical facilitation, but also by allowing earlier embryo-endometrium contact, enabling a higher percentage of embryos to hatch during the critical time-window of implantation. Data on assisted hatching have mainly been published by Cohen et al. The lack of confirming reports may be due to technical difficulties with the procedure itself. Possibly, the potential for embryo damage during transfer is increased after assisted hatching, making a gentle atraumatic transfer necessary (20). Trials from other IVF programmes confirming the efficacy of assisted hatching are needed.

Another micromanipulative technique, the intracytoplasmic sperm injection (ICSI), will almost certainly be introduced in poor responding patients above the age of 40 in order to obtain a

high fertilization rate of the few ova obtained. So far no reports on this subject have been published.

Embryo transfer policy

The number of embryos to be transferred in IVF is still a controversial issue. Replacement of a higher number of embryos enhances the chance to obtain a clinical pregnancy in IVF treatment (23, 50, 51). A pregnancy rate of 55% has been reported by Nijs et al. (51) after replacement of four embryos. On the other hand, transfer of a higher number of good quality embryos leads to an increased number of twin and triplet pregnancies (52). Triplet pregnancies should be avoided because of the social and medical risks for the mother and children (53). Several authors advised replacement of two embryos only in order to avoid triplet pregnancies (21, 22, 51). In these studies the restricted embryo replacement policy did not affect the pregnancy rates significantly. However, in two of these three studies the investigators used an age limit for this policy. Staessen et al. (21) replaced two embryos only when the following criteria were fulfilled: first attempt for IVF, less than 37 years old, and good embryo development. Vauthier-Brouzes et al. (22) used the age limit of 35 years as one of the criteria. Nijs et al. (51) mentioned no age limit for replacement of the maximum number of two embryos, but in a later paper Nijs (52) mentioned an ongoing trial with patients > 35 recieving two or three embryos in an alternate manner. Although replacement of only two embryos appears to be advisable, a lower embryo implantation rate in higher age groups has been reported (23, 24). Therefore, the age of the patient could be regarded as one of the parameters before a decision on the number of embryos to be transferred is taken. On the other hand, a report by Arthur et al. (54) on 1190 consecutive IVF cycles showed no significant difference in the embryo implantation rates in different age groups. Replacement of three or four embryos in older patients, although possibly indicated to enhance the chances for a pregnancy, will inevitably lead to high-order pregnancies in some cases and should therefore be discouraged.

Oocyte donation

Lutjen et al. (55) reported the first human pregnancy after implantation of a donated ovum and proposed the procedure as treatment of infertiliy due to primairy ovarian failure. Since then several indications for this technique have been added. Serhal and Craft (25) extended the use of oo-

cyte donation (OD) to infertile women with a poor response to standard ovulation induction, repeatedly failed IVF or gamete intrafallopian transfer and to infertile women over forty years of age who are still menstruating. Sauer et al. (56) and Abdalla et al. (57) reported the use of OD as treatment for women over 40 years old with ovarian failure. In the past few years several reports have appeared of programmes accepting recipients for OD in even higher age groups (58-60). Oocyte donation in order to achieve pregnancies in post-menopausal women is controversial and ethical aspects have been discussed by Edwards (61) and Antinori (62).

A survey in the United States showed that 27% of the clinics providing OD programmes had no upper age limit for recipients, while many clinics (31%) excluded recipients above 40 years of age from their programmes (63).

It has been argued that pregnancy obtained by OD in older women, may expose the recipient to an unacceptable high risk. Kirz et al. (64) concluded in a retrospective study that women above 35 years of age, who are delivered in a modern tertiary care centre, may however be at no higher risk of adverse outcome than younger parturients aged 20-25 years. Analysis of data from 511 pregnancies in women aged 40 or older by Spellacy et al. (65) showed that older women of low parity and normal weight can expect a good pregnancy outcome. A retrospective study of the outcome of pregnancy in 100 primiparae aged 40 years and more by Brassil et al. (66) showed no increase in maternal morbidity or perinatal mortality rate. Berkowitz et al. (67) studied the outcome of pregnancy in first births in a hospital-based cohort study of 3917 patients with a singleton pregnancy. Although women after the age of 35 appeared to have higher rates of complications of pregnancy and delivery, their risk of a poor neonatal outcome is not appreciably increased. The selection of healthy women as acceptors of donated oocytes may reduce risks (28). Several protocols for screening of recipients have been reported (58,60).

The results of OD programmes have provided important information on the main factors influencing female fecundity at a later age. Ageing of the uterus has been considered an important cause of infertility. Goswamy et al. (68) presented data from an ongoing study to test the hypothesis that poor uterine perfusion is a cause of failure of the implantation of embryos. Feldberg et al. (4) suggested that the high abortion rate in women after the age of 40 years may also be due to the ageing uterine environment. In one of the first larger studies on OD Abdalla et al. (57) found a decrease in pregnancy rate in recipients aged 40-49 years. In their study the authors could not find an adverse effect of increasing age of the donors on the pregnancy rate. However, in only 7 out of 100 donation cycles the oocytes were obtained from donors aged 35 years or more. Other studies only partly confirm the contribution of aging of the uterus to the decline in fecundity with increasing age. Levran et al. (69) found a significantly lower pregnancy rate in recipients aged over 33 years in 169 OD cycles. In the same study increasing age of the donor was related to a higher miscarriage risk. Yaron et al. (26) retrospectively analysed results of standard IVF and OD in pa-

tients < 40 and ≥ 40 years of age. In standard IVF, clinical pregnancy rates were significantly lower in the older patients: 12.9% versus 23.8%. In OD, with no difference in donor age and all donors ≤ 32 years old, pregnancy rates were also significantly lower in patients above 40 years: 21.2% versus 29.3%. The authors concluded that the decrease in endometrial receptivity with age is responsible for the higher rate of implantation failure in older women. However, a pregnancy rate of 21.2% after OD in patients aged ≥ 40 years compared to 12.9% after standard IVF in the same group suggests an effect of ageing of the oocytes.

Indeed, ageing of the ovaries appears to affect fecundity more than ageing of the uterus. It has been demonstrated that when oocytes are obtained from younger donors, the clinical pregnancy rate and the embryo implantation rate are high, irrespective of the recipient's age (25, 27, 57, 70). Navot et al. (27) reported results of an oocyte donation programme with donors and recipients sharing oocytes from the same cohort. Thirty-five women, aged 40 years or more (mean age 42.7 ± 0.3), who had failed to concieve with their own oocytes recieved oocytes donated by 29 young women (mean age 33.4 ± 0.7) undergoing IVF. Clinical pregnancy rates (33% and 40%) and delivery rates (23% and 30%) did not differ between donors and recipients. The authors stated that the fact that all the viable pregnancies with ovum donation took place after the failure of conception with own oocytes excludes a negative effect of ageing on endometrial vasculature and uterine blood supply. It thus appears that decreased embryo viability is the main cause of IVF failure in patients above 40 years of age.

The establishment of artificial cycles is to be preferred in OD programmes. Down-regulation of the pituitary gland with GnRH-a until serum levels of gonadotrophins reach basal levels is followed by giving oral estradiol valerate to prepare the uterus. After 15 days, progesterone is added in order to establish a secretory endometrium. Using this scheme for steroidal support of the uterus Edwards et al. (70) found acyclic women to be more fecund than cyclic women in an OD programme. Amenorrhoeic women concieved more frequently, and to higher ages. Serhal and Craft (25) and Sauer et al. (56) also found high pregnancy rates after OD in acyclic women, but the number of patients in their studies was limited. A large series of OD in amenorrhoeic women after steroidal support of the uterus was reported by Yaron et al. (26). In 219 OD cycles 68 pregnancies were achieved, giving a pregnancy rate of 31.1%. This pregnancy rate was significantly higher than the 19.7% pregnancy rate in the group of 239 cyclic women. It can therefore be concluded that amenorrhoea maintains a favourable uterus. It has even been suggested that a period of induced amenorrhoea might improve the uterine environment in premenopausal women (70).

In OD programmes the miscarriage rate is directly related to the age of the donor and not to

the age of the recipient. Serhal and Craft (25) found only one miscarriage in 12 women older than 42 years who achieved a pregnancy after OD. Navot et al. (71) reported a 16.7% (2 of 12) pregnancy loss in women aged over 40 who recieved oocytes from a donor group with a mean age 30.2 ± 4.9 years. Abdalla et al. (72) described an increasing miscarriage rate with higher donor age. When the age of the donor was 35-39 years, the miscarrige rate for recipients was 45%. Considering the higher implantation rates and lower miscarriage rates after OD with oocytes from younger women, OD has been proposed as an alternative for patients with a poor prognosis for IVF treatment (25-27).

Conclusions

The patient's age, although an indicator of fecundity, should not be the main parameter for acceptance of patients for IVF treatment. Results of prestimulation tests like the determination of basal FSH level and the clomiphene citrate stimulation test are more useful for counselling patients before IVF treatment is started.

Several proposals have been made to improve the results of IVF treatment in patients above 35 years of age. Controlled ovarian hyperstimulation in older patients with a poor ovarian response is still a controversial issue. Contradicting results have been published about the relation between dosage of gonadotrophins used and oocyte yield. When a poor response to ovarian hyperstimulation is expected, GnRH-a should only be used in the short flare-up protocol. Assisted hatching of embryos with a thick zona pellucida has been reported to improve the embryo implantation rate. More studies from other IVF programmes are needed to determine whether this technique is beneficial for poor prognosis patients in higher age groups. Replacement of more than two embryos in patients above 35-40 years of age may be indicated in order to enhance the chances for a pregnancy. However, the embryo implantation rate in this patient group in an IVF programme should be taken into consideration, since triplet and quadruplet pregnancies have to be prevented. Oocyte donation, although controversial in post-menopausal women, seems an attractive alternative for IVF patients above the age of 40 who have failed to concieve with their own oocytes. The use of oocytes obtained from younger donors gives good results and has illustrated the relatively minor role of ageing of the uterus in the decline of female fecundity with rising age. Better results in OD programmes are obtained with artificial cycles.

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Chapter 5

The ovarian response as a predictor for successful in vitro fertilization treatment after the age of 40 years

Fertility and Sterility 1996; 66:969-73

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Summary

To determine whether age or response to controlled ovarian hyperstimulation (COH) is a better predictor of IVF outcome in women ≥ 40 years, a retrospective analysis of 2588 consecutive cycles in a transport IVF program was carried out. The correlation between treatment outcome and age, and response to COH was analyzed. The main outcome measure was pregnancy.

Results: The incidence of poor ovarian response rises significantly with increasing age. Analysis of all cycles showed a significant decrease in clinical and ongoing pregnancy rate for women ≥ 40 years. Analysis of cycles with a good ovarian response showed no statistically significant differences for these parameters between women ≥ 40 and those younger. A logistic regression analysis on pregnancy showed that ovarian response contributes more to the prediction of pregnancy than age.

Conclusions: Patients aged ≥ 40 years with a good response to COH have a good prognosis for IVF treatment. The age limit for acceptance of patients should not be set at 40 years. Instead, the response to COH can be used to predict candidates likely to have a successful IVF outcome.

Introduction

In vitro fertilization treatment is time-consuming and stressful for patients. Assisted reproductive techniques are expensive and considerable debate exists about who will benefit from the procedures, and to what extent health insurances should pay for them. For these reasons

appropriate patient selection is mandatory and a clear indication for treatment must exist. Difficulty arises when there is an indication for IVF, but the chances of a successful treatment outcome are considered low. Poor IVF results have been reported in patients above 40 years of age (1-4). This has led to age limits for acceptance of patients in many programmes. The general opinion in IVF programmes in The Netherlands is that patients aged 40 years or older should not be offered treatment. Setting a strict age limit is debatable. The determination of basal follicle-stimulating hormone (FSH) levels (5) and clomiphene citrate-stimulated FSH levels (6) have been shown to be better predictors of IVF outcome than age. However, pregnancies have been reported in patients with very high basal FSH levels (5,7), indicating that these endocrine screening tests are more useful for patient counselling than for strict patient selection. We hypothesized that response to COH might be a good predictor of IVF outcome that can be used prior to follicle aspiration. To test this hypothesis, we analyzed the correlation between IVF outcome and age and response to COH in 2588 consecutive cycles of IVF.

Materials and methods

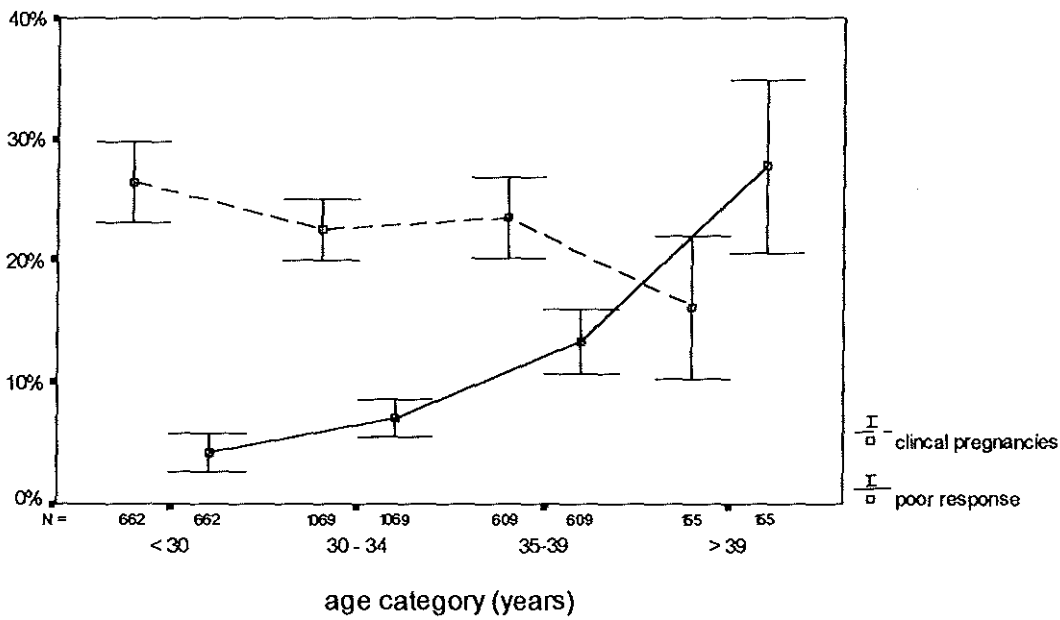
From the period 1989-1994, the results of 2588 consecutive started IVF cycles, carried out in the transport IVF programme at the Zuiderziekenhuis, were available for analysis. This programme, carried out in cooperation with the IVF laboratory at the University Hospital Dijkzigt, has been described elsewhere (8). The monitoring of ovarian response during COH was carried out with transvaginal ultrasound measurements only (9). No strict age limit for acceptance of patients was used. Determination of basal FSH was not done on a routine basis when patients had a regular menstrual cycle. Cancellation of started IVF cycles was considered in cases with disappointing follicular development during COH. However, strict criteria for cancellation were not used. When a better ovarian response in a following cycle was not expected, and the patient felt confident to proceed to oocyte retrieval, treatment was continued despite the relatively poor prognosis. Ninety-three out of 2588 cycles were cancelled prior to follicle aspiration. In 67 cases the reason for cancellation was a poor ovarian response. Follicle aspiration was carried out in 2495 cycles. A poor ovarian response, defined as the development of ≤ 3 follicles measuring ≥ 15 mm on the day of human chorionic gonadotrophin (HCG) administration, occurred in 227 out of these 2495 cycles.

For initial analysis four different age categories were introduced: patients aged < 30 years (group 1), 30-34 years (group 2), 35-39 years (group 3) and ≥ 40 years (range 40-44, group 4). The results of completed IVF cycles in the different groups were analyzed for number of patients

proceeding to embryo transfer, oocyte yield, number of embryos replaced and clinical, ongoing and multiple pregnancies achieved. The embryo implantation rate for the various age categories was calculated. To analyse the impact of treatment continuation in IVF cycles with a poor prognosis on the overall results of our IVF programme, cycles with a poor ovarian response and cycles with a good response were evaluated separately. To test the hypothesis that the ovarian response to COH is a better predictor for pregnancy than the patient's age, a logistic regression analysis on pregnancy was carried out with age (< 40, ≥.40) and ovarian response (good, poor) as dichotomous variables.

A clinical pregnancy was defined by a positive urinary test 18 days after oocyte retrieval combined with the finding of a gestational sac 2 weeks later. A pregnancy was considered to be ongoing when fetal heart motion was observed after 12 weeks of amenorrhoea. The results of cryopreservation/thaw cycles were not included in this analysis. For statistical analysis a logistic regression analysis was carried out, and χ^2 test for trend and Student's t-test were used where appropriate. $P < 0.05$ was taken as level of significance.

Figure 1. Percentages of poor ovarian response and percentages of clinical pregnancies (95% confidence intervals) in completed IVF cycles.



Results

The results of all started IVF cycles in the four age groups, irrespective of the ovarian response during COH are shown in table 1. Out of the 2588 IVF cycles started, 2495 (96%) proceeded to oocyte retrieval. The chance for oocyte retrieval and a following embryo transfer decreased with rising age. Lower clinical and ongoing pregnancy rates were found with rising age: $P = 0.02$ and $P = 0.007$ respectively. The embryo implantation rate appeared to decrease with rising age as well: $P=0.001$. The oocyte yield did not differ between groups 1 and 2, but the differences found between groups 2 and 3, and between groups 3 and 4 were statistically significant: $P = 0.001$ for both. The number of embryos replaced per embryos transfer in group 3 and 4 were the same.

Table 1: Results of Started IVF Cycles in the Four Age Groups.

Age group	1	2	3	4	*P* value
Age (y)	<30	30-34	35-39	≥40	
Started cycles	674	1099	646	169	
Cancelled, poor response *	5 (0.8)	20 (1.8)	29 (4.5)	13 (7.7)	0.0001†
Oocyte retrievals *	662 (98)	1069 (97)	609 (94)	155 (92)	0.0001†
Completed, poor response ‡	28 (4)	75 (7)	81 (13)	43 (28)	0.0001†
Embryo transfers ‡	562 (85)	871 (81)	502 (82)	109 (70)	0.002 †
Clinical pregnancies ‡	175 (26)	241 (23)	143 (23)	25 (16)	0.02 †
Ongoing pregnancies ‡	144 (22)	200 (19)	113 (19)	17 (11)	0.007 †
Multiple pregnancies §	51 (29)	61 (25)	34 (24)	2 (8)	NS ‖
Embryo implantation rate (%)	17.0	14.2	12.7	9.2	0.001†
Oocytes retrieved ¶	10.8 ± 7.1	10.2 ± 7.2	8.5 ± 7.2	5.7 ± 4.6	0.001**
Embryos transferred ¶††	2.4 ± 0.7	2.6 ± 0.9	2.8 ± 1.0	2.7 ± 1.0	NS **

* Values in parentheses are percentages per started cycle.

† χ^2 for trend.

‡ Values in parentheses are percentages per oocyte retrieval.

§ Values in parentheses are percentages of clinical pregnancies.

‖ NS not significant.

¶ Values are means ±SD.

** Student's t-test for difference group 3 and 4.

†† Per embryo transfer.

Fewer embryos were replaced in group 1 when compared with group 2 ($P = 0.03$), and in group 2 compared with group 3 ($P = 0.001$). For further illustration the percentages of cycles with a poor ovarian response and the percentages of clinical pregnancies in completed IVF cycles in the four different age categories are shown in figure 1.

The results obtained in IVF cycles with development of ≤ 3 follicles measuring ≥ 15 mm on the day of HCG administration are shown in table 2. Except for the percentage of embryo transfers ($P = 0.014$), no statistically significant differences were found between the age categories. The 43 oocyte retrievals in group 4 resulted in one ongoing pregnancy only.

Results obtained in the four age groups for completed cycles with a good ovarian response to COH are shown in table 3. No statistically significant differences were found between the age groups for percentages of patients who proceeded to embryo transfer and for clinical and ongoing pregnancy rates. The embryo implantation rate decreased significantly with increasing age. Statistically significant differences for oocyte yield were found between groups 2 and 3 ($P = 0.001$), and between groups 3 and 4 ($P = 0.001$). With rising age more embryos were replaced per embryo transfer, giving significant differences between groups 1 and 2 ($P = 0.001$) and between groups 2 and 3 ($P = 0.001$).

Table 2: Results of Completed IVF Cycles with a Poor Ovarian Response.

Age group	1	2	3	4	P value
Age (y)	<30	30-34	35-39	≥40	
Oocyte retrievals	28	75	81	43	
Embryo transfers *	19 (68)	53 (71)	52 (64)	19 (44)	0.014†
Clinical pregnancies *	5 (18)	8 (11)	11 (14)	3 (7)	NS†
Ongoing pregnancies *	5 (18)	6 (8)	8 (10)	1 (2)	NS†
Embryo implantation rate (%)	18.7	11.1	14.3	9.4	NS†
Oocytes retrieved ‡	2.1 ± 1.1	1.9 ± 0.8	1.8 ± 1.1	1.5 ± 1.1	NS§
Embryos transferred ‡‖	1.7 ± 0.7	1.5 ± 0.6	1.6 ± 0.7	1.7 ± 0.7	NS§

* Values in parentheses are percentages per oocyte retrieval.

† χ^2 for trend.

‡ Values are means ± SD.

§ Student's t-test for differences between groups 3 and 4.

‖ Per embryo transfer.

The percentages of clinical pregnancies in relation to age (age as a categorical variable dichotomized at 40) and ovarian response (good, poor) are shown in table 4.
The results of a logistic regression analysis on pregnancy with the dichotomous variables ovarian response (good, poor) and age (< 40, ≥ 40) are shown in table 5.

Table 3: Results of Completed IVF Cycles with a Good Ovarian Response.

Age group	1	2	3	4	P value
Age (y)	<30	30-34	35-39	≥40	
Oocyte retrievals	634	994	528	112	
Embryo transfers *	543 (87)	818 (82)	450 (85)	90 (80)	NS†
Clinical pregnancies *	170 (27)	233 (23)	132 (25)	22 (20)	NS†
Ongoing pregnancies *	139 (22)	194 (20)	105 (20)	16 (14)	NS†
Embryo implantation rate (%)	17.0	14.3	12.6	9.2	0.001†
Oocytes retrieved ‡	11.2 ± 7.0	10.8 ± 7.1	9.5 ± 7.2	7.3 ± 4.5	0.001§
Embryos replaced ‡‖	2.5 ± 0.7	2.6 ± 0.9	2.9 ± 0.9	2.9 ± 1.0	0.001§

* Values in parentheses are percentages per oocyte retrieval.
† χ^2 for trend.
‡ Values are means ± SD.
§ Student's t-test for differences between groups 3 and 4.
‖ Per embryo transfer.

Table 4: Clinical Pregnancies after IVF Treatment in Relation to Age (< 40 years, ≥ 40 years) and Ovarian Response (Good, Poor).

Age	Response	Oocyte retrievals	Clinical pregnancies *
< 40	Good	2156	535 (24.8)
< 40	Poor	184	24 (13.0)
≥ 40	Good	112	22 (19.6)
≥ 40	Poor	43	3 (7.0)

* Values in parentheses are percentages per oocyte retrieval

Table 5: Logistic Regression Model.

	Regression coefficient (b)	Standard error	P value	Odds ratio	95% confidence interval
Age	-0.36	0.23	0.12	0.70	0.44-1.09
Ovarian response	-0.83	0.21	0.0001	0.44	0.29-0.66

Discussion

For appropriate patient selection and counselling, predictive tests for a patient's individual performance in IVF treatment are needed. Tests with a high predictive value could give patients and physicians realistic expectations of the chances for pregnancy. Ideally, these tests should also allow identification of patients who are destined for treatment failure and thus should not undergo the procedure. However, the methods used for identification of patients with a poor prognosis for successful outcome of IVF treatment are controversial. It is recognised that advanced age is correlated with reduced chances for pregnancy after IVF. This has led to the use of age limits for IVF treatment. Strict age limits for IVF are debatable. Screening endocrine tests, like the determination of basal (5) and clomiphene citrate-stimulated (6) FSH levels have been proposed and proved to have a better predictive value for IVF treatment outcome than the patient's age. A correlation between higher basal FSH levels and lower pregnancy rates after IVF has been reported. Toner et al. (5) found no ongoing pregnancies in patients with a basal FSH > 25 mIU/mL. However, the same authors reported an ongoing pregnancy rate of 15% for patients with a basal FSH of 20 mIU/mL. Licciardi et al. (7) found no pregnancies in patients having cycle day 3 FSH levels of > 17 mIU/mL and estradiol (E_2) > 45 pg/mL, but in patients with E_2 levels ≤ 45 pg/mL and a basal FSH > 17 mIU/mL, an ongoing pregnancy rate of 16.7% was reported. Apparently, in patient groups with a considerable elevation of basal FSH, pregnancy rates are found that can still be considered acceptable. These findings indicate that screening endocrine prestimulation tests are useful for patient counselling, but should be used with care for patient selection. Screening tests will pick up some patients with very high FSH levels who could be predicted not to respond to ovarian hyperstimulation, but moderate FSH elevations should not be a reason to withhold therapy. It therefore seems appropriate to use the outcome of COH as an indicator for success. In this way the decision whether to complete IVF treatment, including the more stressful and expensive part of IVF treatment: oocyte retrieval, laboratory phase and embryo transfer, is further individualized. When considering the cost of IVF treatment, differences

between countries should be kept in mind. Especially the prices of the gonadotrophins can vary considerably.

In the IVF programme at the Zuiderziekenhuis no strict age limit is used and no standard endocrine screening tests are carried out. Premenopausal patients with an indication for IVF treatment are given an IVF trial. When during COH a poor ovarian response occurs, the patient is informed about the relatively poor prognosis, and, if the patient so chooses, follicle aspiration is carried out. The definition of poor response used was in line with criteria mentioned by other authors (10-12). Of all started cycles only 2.7% were cancelled because of a poor ovarian response. As a result, a relatively large group of patients with a poor ovarian response to COH proceeded to follicle aspiration. This enabled us to test the hypothesis that ovarian response is a better predictor for successful IVF outcome than age.

Analysis of the treatment outcome of all IVF cycles showed that the rates for clinical and ongoing pregnancies in higher age groups were significantly lower. To determine whether this was caused by a high proportion of IVF cycles with a poor ovarian response in the higher age groups, we distinguished between poor and good responders to COH and evaluated the results in the two groups separately. As expected, the results of the poor responders show that poor ovarian response is associated with a poor treatment outcome. Continuation of treatment seemed worthwhile in patients below 30 years of age since in this group 28 oocyte retrievals led to 5 ongoing pregnancies, giving an 18% ongoing pregnancy rate. However, between 30 and 40 years of age the results were poor. There appeared to be hardly any justification for continuation of treatment for patients with a poor ovarian response aged $\geq$ 40. Forty-three oocyte retrievals led to only one ongoing pregnancy. In patients aged $\geq$ 40 with a good ovarian response to COH, the outcome of IVF treatment did not differ significantly from the results in the younger age groups. The oocyte yield was smaller, and the embryo implantation rate lower with rising age, but the ongoing pregnancy rate did not decline significantly. Rising age correlated with an increasing incidence of poor ovarian response. The percentages of poor responders found in the four age groups corresponded well with the discharge rates during stimulation reported by Wood et al. (13).

A logistic regression analysis on pregnancy with ovarian response (good, poor) and age (<40, $\geq$40) as dichotomous variables confirmed that ovarian response is a better predictor for IVF treatment outcome than age. When age of the female partner and ovarian response were included in the logistic regression, the likelihood of pregnancy with poor ovarian response was 0.44, (95% confidence interval 0.29-0.66) (P=0.0001), and the likelihood of pregnancy over the age of 40 was 0.70, (95% confidence interval 0.44-1.09) (P=0.12).

In conclusion; this study shows that ovarian response is a better predictor for successful IVF treatment than age. Patients aged $\geq$ 40 with a good ovarian response to COH have a good

prognosis for IVF treatment. Therefore, the age limit for IVF treatment should not be set at 40 years. When ≤ 3 follicles with a diameter ≥ 15 mm develop during COH, cancellation of the IVF cycle is justified, especially in the older patients. The result of COH can be used to counsel and select patients before the more stressful and expensive phase of IVF treatment starts.

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Chapter 6

Treatment policy after poor fertilization in the first IVF cycle

Submitted for publication

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Summary

Patients with poor fertilization (PF = fertilization of ≤ 20% of all oocytes) in their first IVF cycle were followed in the subsequent cycle to assess the chance of recurrence of this phenomenon. In cases of PF, a differentiation was made between total fertilization failure (TFF), and low fertilization rate (LFR) defined as fertilization of > 0% and ≤ 20% of all oocytes. After analysis of the relation between occurrence of TFF and the number of oocytes retrieved, TFF was defined as fertilization failure in cycles with ≥ 4 oocytes obtained. Based on the relation between the fertilization rate and the number of motile sperm cells/mL semen in the first cycle, patients were divided into 3 subgroups with different sperm parameters; group 1: < $5x10^6$ motile sperm cells/mL, group 2: 5-$19x10^6$ motile sperm cells/mL, and group 3: ≥ $20x10^6$ motile sperm cells/mL in the ejaculate. Patients with a TFF or LFR in the first cycle who continued treatment, were followed in the subsequent cycle. The chance of occurrence of TFF or LFR in the second cycle in the 3 groups was assessed. The recurrence rate of TFF was high: 45 - 70%, and PF occurred in 50 - 75% of all cases. Similarly, after LFR in the first cycle, TFF and PF frequently occurred in the second cycle: 37% and 50% respectively.

In conclusion: In our population, both TFF and LFR in the first IVF cycle has a high chance of recurrence in the subsequent cycle. It appears that TFF is not a separate entity, but the severest form of PF. Consequently, in our population, ICSI should be considered after PF in the first cycle, especially in cases with a small oocyte yield.

Introduction

The incidence of total fertilization failure (TFF) and its predictive significance for subsequent IVF cycles has been reported by several authors (1-5). In contrast, the incidence of low fertilization rate (LFR) in an IVF cycle and its significance for the treatment prognosis has not been given much attention. Apparently, when after LFR one or more embryos are available for replacement, and, consequently, a true chance for pregnancy exists, the IVF attempt is seen as successful. However, if TFF and LFR are both expressions of a defective interaction between oocyte and sperm cell, the recurrence rate in a subsequent cycle and the treatment prognosis of LFR and TFF could be comparable.

The chance of recurrence of TFF in a subsequent cycle as reported by others is approximately 20%, irrespective of the indication for IVF (1-5). Because of this low recurrence rate, it has been advised to undertake 2 more IVF attempts before reverting to other therapeutic options like the use of donor sperm or micromanipulative techniques (3, 5). However, with the introduction of intracytoplasmic sperm injection (ICSI), a micromanipulative technique has become available that has proved to be a good alternative after TFF. For ICSI a TFF rate of 3% only has been reported (6).

In this study the chance of successful fertilization in a subsequent IVF cycle after TFF or LFR in the first cycle is assessed. The aim of the study was to investigate whether TFF and LFR in the first IVF cycle are indications for assisted fertilization in following cycles.

Materials and methods

Patients with poor fertilization (PF = fertilization of $\leq 20\%$ of all oocytes) in their first IVF cycles who continued treatment, were followed in the subsequent cycle. In cases of PF, a differentiation was made between TFF and LFR, defined as fertilization of $> 0\%$ and $\leq 20\%$ of all oocytes. In IVF cycles with a small oocyte yield, TFF can be seen as a chance occurrence in a substantial number of cases. Consequently, to define TFF, an analysis of the relation between the chance of occurrence of TFF and the number of oocytes retrieved was carried out. As reported previously, the average fertilization rate per oocyte in the population of the transport IVF programme is 50% (7). The a priori chance of TFF in relation to the number of oocytes retrieved is indicated in figure 1. The actual incidence of TFF in the population studied is also indicated in this figure. Based on the data obtained, TFF was defined as fertilization failure in cycles with ≥ 4 oocytes retrieved.

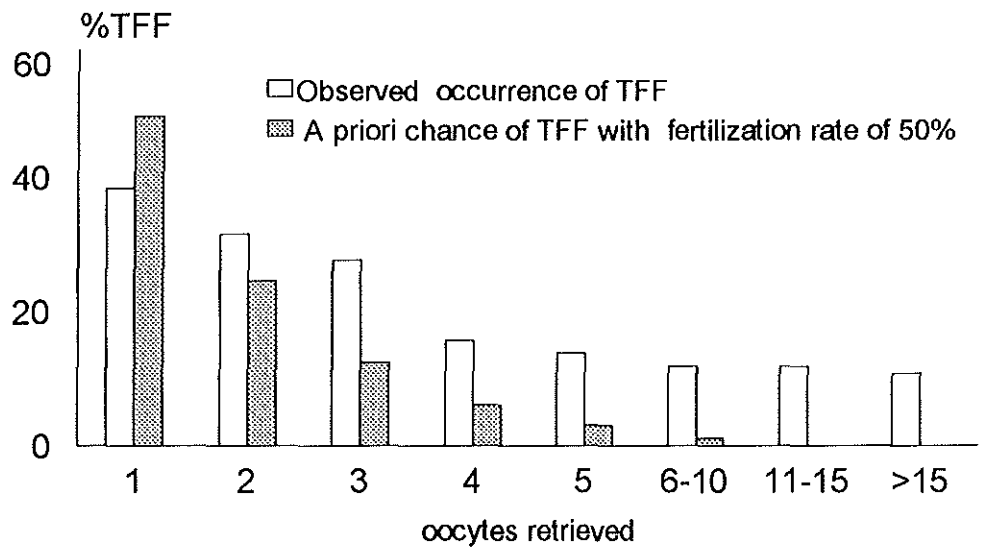
To divide patients into subgroups with different chances of fertilization, the relation between the chance of an oocyte being fertilized and the number of motile sperm cells/mL in the semen used, was analysed for 1211 consecutive first IVF cycles. Three subgroups were defined for separate analysis.

In 1049 out of 1211 patients ≥ 4 oocytes were retrieved in the first IVF cycle. Of these, 207 had a poor fertilization in the first cycle, and 119 proceeded to a second attempt. In the second IVF cycle, 3 patients had < 4 oocytes retrieved and were excluded from the study, leaving 116 patients for further analysis. The second cycles of the patients in the 3 subgroups with TFF or LFR in the first IVF cycle were analysed separately, and the chance of recurrence of poor fertilization in the second cycle was studied.

Results

The theoretical incidence of TFF as a chance occurrence, and the actual incidence of TFF in relation to the number of oocytes retrieved in the first IVF cycle, are shown in figure 1.

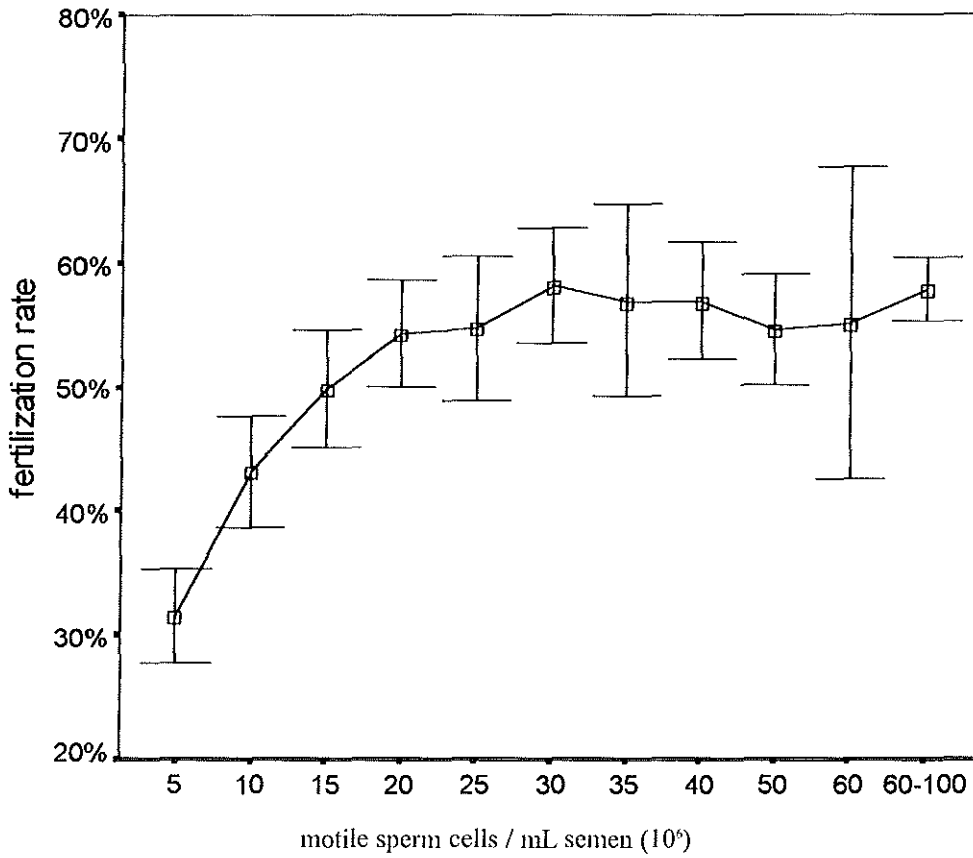
Figure 1.



As the number of retrieved oocytes increases, the incidence of TFF as the result of chance occurrences decreases. The observed incidence in cycles with 4 oocytes retrieved was 16%, while

the theoretical incidence of TFF in these cycles was 6%. For further analysis TFF in cycles with ≥ 4 oocytes was considered as indicative of a defective interaction between oocyte and sperm cell. Consequently, all patients with < 4 oocytes retrieved in the first IVF cycle were excluded from further analysis.

Figure 2. Relation between fertilization rate and the number of motile sperm cells/mL semen.



The relation between the chance of fertilization per oocyte and the number of motile sperm cells/mL semen (median and 25-75 percentiles) is shown in figure 2. Based on this graph 3 subgroups were distinguished; group 1: < $5x10^6$ motile sperm cells/mL, group 2: 5-$19x10^6$ motile sperm cells/mL, and group 3: ≥ $20x10^6$ motile sperm cells/mL.

The incidences of TFF in the first IVF cycle and the chance of recurrence of TFF and occurrence of LFR in the subsequent cycle are shown in table 1. The last column of the table shows the cases with poor fertilization (PF). The recurrence rate of TFF in the subsequent cycle was high in the 3 groups. Poor fertilization occurred in 60% of all cycles following TFF.

Table 1. Total Fertilization Failure in the First Cycle and Poor Fertilization in the Second Cycle in the 3 Groups.

	TFF 1st cycle (*)	Continued (†)	Second cycle		
			TFF (‡)	LFR (‡)	PF (‡)
Group 1 (n=196)	39 (20%)	20	14 (70%)	1 (5%)	15 (75%)
Group 2 (n=232)	46 (20%)	20	8 (40%)	5 (25%)	13 (65%)
Group 3 (n=621)	51 (8%)	38	17 (45%)	2 (5%)	19 (50%)
Total (n=1049)	136 (13%)	78	39 (50%)	8 (10%)	47 (60%)

* Percentage of TFF in the group in the first cycle.

† Patients with < 4 oocytes in the second cycle (n = 3) were excluded.

‡ Percentage of patients who continued after TFF in the first cycle.

The results of subsequent cycles of patients with a low fertilization rate in the first cycle are shown in table 2. Total fertilization failure in a second cycle occurred frequently (37%) after LFR in the first IVF attempt. Poor fertilization occurred in 50% of all subsequent cycles.

Table 2. Low Fertilization Rate in the First Cycle and Poor Fertilization in the Second Cycle in the 3 Groups.

	LFR 1st cycle	Continued	Second cycle		
			TFF (*)	LFR (*)	PF (*)
Group 1	8	2	1 (50%)		1 (50%)
Group 2	27	13	6 (46%)	2 (15%)	8 (61%)
Group 3	36	23	7 (30%)	3 (13%)	10 (43%)
Total	71	38	14 (37%)	5 (13%)	19 (50%)

* Percentage of patients who continued after LFR in the first cycle.

Discussion

Although low fertilization rates have been reported as an indication for the use of micromanipulative techniques (8), the incidence of LFR in IVF has not been given much attention. In contrast, the incidence of TFF, and the treatment policy after TFF has been discussed by several authors. The most frequently given advice is to try conventional IVF treatment again for a second or even a third trial without resorting to assisted fertilization using micro-manipulative techniques (3-5). The advice given by these authors is mainly based on the low recurrence rate for TFF found in their studies. However, the definition of TFF used in these studies can be questioned. Failure of fertilization in cycles with only few oocytes retrieved can be seen as a chance occurrence. In many of these cases there might be no abnormality in the interaction between sperm cell and oocyte. Therefore, based on the incidences of TFF in cycles with a low number of oocytes in our study, we excluded patients with < 4 oocytes retrieved in their first IVF cycle from the analysis. Using this approach, we identified a subpopulation of patients with a recurrence rate of TFF in a second IVF cycle of 50% as shown in table 1. This rate is high when compared with other reports (1-5). When the cases of LFR in the second cycle were included, the incidence of PF in the second cycle was 60%. The introduction of subgroups with different sperm parameters showed a comparable poor prognosis for all 3 groups.

The results shown in the tables 1 and 2 appear to indicate that there is no essential difference between the mechanisms of TFF and LFR. After LFR in the first cycle 37% of patients had a TFF in the subsequent cycle, and 50% of the patients who continued with IVF treatment had a PF in the second cycle. It seems that in the population studied LFR in the first cycle has a similar poor prognosis as TFF. Apparently, TFF should not be regarded as a separate entity in IVF.

With the availability of intracytoplasmic sperm injection (ICSI) the therapeutic options for defective interaction between sperm cell and oocyte have improved considerably. The results of ICSI are good when compared with other micromanipulative techniques like subzonal insemination (9) or partial dissection of the zona pellucida (10). The results obtained in the population studied point out that ICSI might be indicated after TFF in the first IVF cycle. The high recurrence rate of LFR shows that the use of ICSI could even be considered after LFR in the first cycle, especially when few oocytes are expected to be retrieved.

In conclusion, in our population total fertilization failure and low fertilization rate both have a high recurrence rate. The use of ICSI should therefore be considered after poor fertilization in the first cycle.

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2.
Procedure-related clinical complications

Chapter 7

The incidence of major clinical complications in a transport IVF programme

Human Reproduction Update 1996; 2:345-53.

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Summary

Four different major clinical complications are identified in a retrospective analysis of 2495 IVF cycles resulting in oocyte retrieval. The severe form of ovarian hyperstimulation syndrome (OHSS) occurred in 18 patients, giving a prevalence for this complication of 0.7%. Seven (39%) out of these 18 patients were previously diagnosed as having polycystic ovaries. Eleven patients were admitted with moderate OHSS. Adnexal torsion was diagnosed in two patients. Ovariectomy was considered necessary in both cases. Complications of the transvaginal procedure occurred in 7 cases (0.3%); one patient had an acute apendicitis with puncture holes in the appendix, 6 patients were admitted shortly after oocyte retrieval with a pelvic inflammatory disease (PID). Thirteen out of the 624 pregnancies obtained were ectopics, giving an ectopic pregnany rate of 2.1%. It is concluded that serious clinical complications of IVF treatment are rare. However, patients should be counselled for the occurrence of serious procedure-related complications before entering an IVF programme.

Introduction

In vitro fertilization treatment is an elective procedure. Clinical complications caused by the procedure are therefore always iatrogenic. Although complications are reported to occur infrequently, counselling of patients on possible complications of IVF treatment is necessary before informed consent can be signed. The incidence of complications appears to differ between programmes and could depend on various factors like: methods used for controlled ovarian

hyperstimulation (COH), criteria used for cancellation of cycles, and techniques used for follicle aspiration and embryo transfer. Therefore it seems advisable to inform patients about the incidence of clinical complications in the very IVF programme they are about to join. To obtain data concerning the incidence of clinical complications leading to hospital admission in our transport IVF programme a retrospective analysis is carried out. In the population studied, four different major complications are identified: ovarian hyperstimulation syndrome (OHSS), adnexal torsion, pelvic inflammatory disease (PID) and ectopic pregnancy. The occurrence of high-order pregnancies is not discussed since this will be the subject of a separate paper. Detailed reports have been published on the occurrence of a hepatitis-B infection related to the IVF-laboratory procedure (1). Publications on assisted reproduction including information concerning complications are reviewed, and the findings of our retrospective study are compared with these reports.

Materials and methods

In a retrospective analysis of 2495 consecutive cycles resulting in oocyte retrieval, the incidence of major clinical complications of IVF treatment is assessed. A major complication is defined as a complication resulting from IVF treatment leading to hospital admission. The organizational aspects of our transport IVF programme with satellite clinics has been described previously (2). Patients were accepted for the programme after serological tests (HBsAg, syphilis, human immunodeficiency virus) and routine physical examination. Controlled ovarian hyperstimulation consisted of human menopausal gonadotrophin (HMG) 150-450 IU i.m./day (Humegon®; Organon, Oss, The Netherlands), depending on age, and starting on cycle day 3. The standard starting dose of HMG for patients aged below 35 years was 150 IU/day, above this age 225 IU/day was used. Patients diagnosed as having polycystic ovaries, or known with a previous explosive ovarian response during COH started with 75-150 IU/day. Since the introduction of gonadotrophin-releasing hormone agonists (GnRH-a), the short flare-up regime was used in most cases, starting on cycle day 1 until the day of human chorionic gonadotrophin (HCG) injection. Plasma estradiol (E_2) measurements were not carried out during COH. Monitoring of follicle growth was performed by vaginal ultrasound measurements only. When > 35 developing follicles were seen during monitoring, the cycle was cancelled; in most cases by withholding HCG and continuing GnRH-a to reduce the chance of development of OHSS. Oocyte retrieval was planned 35 hours after i.m. injection of 10 000 IU HCG (Pregnyl®; Organon) on the day the leading follicles reached a diameter of 18-20 mm.

Follicle aspiration was carried out by ultrasound-guided vaginal puncture under local anaesthesia. Preventive antibiotics were not used. Chemical desinfection of the vagina was not used in view of possible harm to the oocytes. Before oocyte aspiration the vagina was soaked twice with sterile isotonic saline solution. The ultrasound transducer was covered with a sterile plastic sheet. The patient was covered with a sterile, single-use surgical sheet, the gynaecologist used sterile surgical gloves. Bilateral ovarian puncture was carried out with the same one-way needle. Evacuated follicles were not rinsed. Luteal support was given with 1500 IU HCG on the day of ovum retrieval (day 0) and on days 3, 6 and 9. When $\geq$ 15 follicles were retrieved, progesterone (200 mg vaginally twice daily; Progestan®; Organon) was prescribed for 15 days. In these cases HCG for luteal support was withheld. Replacement of embryos was carried out 2 to 5 days after oocyte retrieval. Embryos (initially a maximum of four) were replaced to the midcavity position. The amount of culture fluid used varied from 30 - 60 ml. No routine consultation was planned during the first 2 weeks after embryo transfer. Patients were instructed to contact the clinic in case signs and symptoms of possible complications occurred. For the classification of OHSS the classification of Golan (3) was used. All patients with a severe OHSS or with signs of PID were routinely admitted for clinical observation and treatment.

Ovarian hyperstimulation syndrome

The severe form of OHSS was diagnosed in 18 patients, giving a prevalence per oocyte retrieval of 0.7%. Twelve cases were diagnosed as grade 4, 6 cases were grade 5. The median length of hospital stay was 11 days, range 4-25 days. Five patients underwent abdominal paracentesis for relief of symptoms. In one case with extensive pleural effusion, bilateral thoracal drainage was necessary. In 13 out of 18 patients (72%), the IVF cycle resulted in a pregnancy; 2 triplets and 7 twins, giving a multiple pregnancy rate of 69%. Although moderate OHSS was not routinely followed by hospital admission, 11 patients with this condition were admitted. The median duration of admission for moderate OHSS was 8 days, range 4-12 days. Four patients (36%) were pregnant, all multiple pregnancies; 2 triplets and 2 twins.

The ovarian hyperstimulation syndrome is the most frequent complication of COH. Reports of OHSS developing after all methods of ovulation induction have been published (4). In rare cases, the severe form of OHSS can result in a life-threatening condition (5). The characteristic clinical components of OHSS are ovarian enlargement with multiple cyst formation and oedema of the stroma, and an acute fluid shift from the intravascular space to the extravascular compartment, associated with ascites, hydrothorax and generalised tissue oedema. The latter component of the

syndrome is the main cause of the morbidity and mortality related to it. The fluid accumulation in the peritoneal and pleural cavities leads to hypovolemia and hemoconcentration. Without correction, renal perfusion can be modified with resultant oliguria. Increased hemoconcentration may lead to thrombosis and thromboembolism.

Classification

The first classification of the OHSS was introduced by Rabau et al. (6), later modified by Schenker and Weinstein (4). This classification divided the syndrome in three clinical stages: mild, moderate and severe, and six grades. When ovarian stimulation is carried out for assisted reproductive techniques (ART), mild hyperstimulation, allowing recruitment of a cohort of mature follicles, is a primary goal of the treatment. As a result, the 'mild' OHSS, as proposed by Rabau et al., is part of almost every stimulation cycle for IVF. In the absence of clinical symptoms it has no clinical value and should not be seen as a complication. Therefore, Golan et al. (3) proposed a new classification as shown in table 1.

Pathogenesis

The pathogenesis of OHSS is still poorly understood. Several mediators for the pathogenesis have been proposed. Estradiol does not seem to play an important role in the pathogenesis of OHSS. Estradiol is a marker for the ovarian response, not the causative agent in OHSS (7). Pellicer et al. (8) reported moderate to severe OHSS in a woman with a partial 17,20-desmolase deficiency and very low serum E_2 levels.

Polishuk and Schenker (9) stated that the development of ascites is secondary to the increase of capillary permeability of the peritoneum mediated by a vaso-active substance secreted by the ovaries. The rapid ovarian growth requires extensive angiogenesis which itself is associated with an increase in capillary permeability. Frederick et al. (10) showed the agent(s) required for this neovascularization to be present in human follicles measuring > 16 mm diameter by demonstrating angiogenesis in the rabbit cornea after injection of human follicular fluid. Fernandez et al. (11) found high renin-like activity in preovulatory follicular fluid. This, and the demonstration of a correlation between plasma renin activity and severity of OHSS (12), led to the theory that these substances play an important role in the pathogenesis of the syndrome. More evidence was found indicating that activation of the renin-angiotensin-aldosterone system is a primary event in OHSS and not merely the consequence of plasma volume concentration (13). Very high plasma levels of active renin and aldosterone were found in patients with severe OHSS, which were not suppressed when the extracellular compartments were greatly expanded. The

values subsequently declined to normal levels, despite the use of diuretics, suggesting that the renin was of non-renal origin since its production was apparently unaffected by influences which control renal juxtaglomerular secretion.

Table 1. Clinical Classification of Ovarian Hyperstimulation.

Classification	Grade	Symptoms
Mild	1	Abdominal distension and discomfort
	2	Grade 1 + nausea, vomiting and/or diarrhea, ovaries < 12 cm
Moderate	3	Mild + ultrasonic evidence of ascites
Severe	4	Moderate + clinical evidence of ascites and/or hydrothorax or breathing difficulties
	5	All the above + increased blood viscosity due to hemoconcentration, coagulation abnormalities, diminished renal perfusion and function

Prostaglandins are other vasoactive substances proposed as possible mediators in the development of OHSS. However, the administration of indomethacin (14) had no influence on ascites formation in animal experiments. Histamine is another substance that has been evaluated as a possible mediator. Although the use of histamine-receptor blockers in animal studies yielded promising results in reduction of ascites and ovarian size in OHSS, later studies failed to detect a role for histamine in the pathogenesis (15).

Prevalence and factors influencing the incidence of OHSS after ovarian stimulation

In IVF treatment, the development of mild ovarian hyperstimulation, allowing recruitment of a cohort of mature follicles, is a primary goal. The incidence of severe OHSS is relatively rare in patients undergoing ovarian stimulation for IVF when compared with ovulation induction cycles. Schenker and Weinstein (4) reported the occurrence of severe OHSS after ovarian stimulation for ovulation induction in approximately 2% of the cycles. The incidence of severe OHSS after COH ranges from 0.5 to 1.8% (16-21). The prevalence of 0.7% found in our population is comparable with these reports. Puncture and aspiration of the ovarian follicles is apparently an effective way to avoid ovarian hyperstimulation (3).

Several factors have been reported to influence the incidence of OHSS. The importance of polycystic ovaries (PCO) as a risk factor has been stressed (4, 19, 22, 23). Among the 18 patients

with severe OHSS in our population, 7 (39%) had been diagnosed as having polycystic ovaries. A high ovarian response during COH, resulting in a high oocyte yield, is associated with a higher incidence of OHSS (23-25). An increased prevalence of OHSS is seen when GnRH-a is used during COH (19, 26, 27). The administration of ovulatory HCG has been proposed as the most important factor in inducing OHSS (17, 23). The pregnancy rate in OHSS cycles is reported to be 3-4 times higher when compared to cycles without signs of hyperstimulation (26). Oocyte donors in donor programmes have a very low risk of OHSS, probably because of the absence of pregnancy after COH (28). These reports suggest a positive role of endogenous HCG in the development of OHSS. Dahl Lyons et al. (29) differentiated between early OHSS, presenting 3-7 days after HCG administration, and late OHSS, presenting 12-17 days after HCG administration. Early OHSS was predicted by the number of oocytes retrieved and the E_2 concentration on the day of HCG administration, while late OHSS correlated with the number of gestational sacs seen on ultrasound 4 weeks after embryo transfer. The authors concluded that early OHSS was an effect of the HCG administered prior to oocyte retrieval, whereas late OHSS was induced by the rising serum concentration of HCG produced by the early pregnancy. In case a high ovarian response occurs, the use of exogenous HCG for luteal phase supplementation increases the risk of development of OHSS (22).

Prevention of OHSS

Several methods have been proposed to lower the incidence of OHSS. Patients who previously developed OHSS, or who are known to have PCO, should be treated with a low-dose gonadotrophin protocol (19, 22). In our IVF program, patients with PCO are treated with 2 ampoules (150 U) HMG daily, patients who previously had OHSS with 1-2 ampoules. The condition did not recur in the severe form in the same patient.

Estradiol peak level and rate of increase during COH, number and size of follicles developing, and number of oocytes collected are parameters used for prediction of the development of OHSS. In view of this, close monitoring of COH as a method for prevention has been advised (22). However, a considerable overlap of distribution of these values between OHSS and control populations has been found (24). Therefore, the value of intensive monitoring for prevention of OHSS has been debated (30, 31).

Monitoring for impending OHSS is carried out by transvaginal ultrasound examination, frequently combined with measurements of serum E_2 concentration during COH. In case an explosive ovarian response occurs, ovulatory HCG can be withheld and the IVF cycle cancelled. However, precise criteria for impending OHSS and subsequent cancellation of the IVF cycle are lacking. There is wide variation between authors in the E_2 levels above which they advise HCG to

be withheld. Forman et al. (27) withheld HCG at an E_2 serum level > 2000 pg/ml. In these cases the administration of gonadotrophins was stopped and GnRH-a continued. After a period of desensitization follicular stimulation was recommenced with a lower dose of gonadotrophins. Other authors (32) developed less strict criteria, and reported that cycles with E_2 levels $\leq$ 5000 pg/ml need not be cancelled and can proceed to oocyte recovery and embryo transfer. Morris et al. (28) found only one case of OHSS among 10 patients with E_2 concentration > 6000 pg/ml and > 30 oocytes recovered. The authors concluded that the risk of OHSS at high levels of stimulation is lower than previously believed. In contrast, Asch et al. (33) found OHSS in 80% of the patients with peak levels > 6000 pg/ml and >30 oocytes collected. The same authors found no cases of OHSS with serum E_2 levels < 3500 pg/ml, and only one case in a group of 67 patients with levels between 3500 and 6000 pg/ml.

In several IVF programmes monitoring of COH is carried out by transvaginal ultrasound examinations only (2, 30, 34). In these programmes the number of developing follicles is used as criterion to cancel the cycles or proceed to oocyte retrieval. The development of more than 30-35 follicles, corresponding with a serum E_2 level of approximately 6000 pg/ml (33), has been mentioned as criterion for cancellation (25,31).

Besides cancellation of the cycle, several other approaches are possible in cases of impending OHSS. Withholding the ovulatory dose of HCG is one option. It was hypothesized that the longer half-life, higher affinity and longer intracellular effect of exogenous HCG compared to endogenous HCG results in a more extensive luteinization of hyperstimulated ovaries. Therefore, Gonen et al. (35) used the initial flare-up effect of GnRH-a to achieve the final follicle maturation. The discontinuation of gonadotrophin therapy and deferring HCG administration until the plasma E_2 concentration drops below 3000 pg/ml, called "prolonged coasting", has also been proposed (25).

Elective cryopreservation of all embryos of patients at high risk of development of OHSS, to avoid a pregnancy and subsequent production of endogenous HCG in the stimulation cycle, is another option for prevention. It has been reported that this procedure reduces the severity of OHSS, but not the incidence (23). However, a lower incidence of OHSS after cryopreservation of all embryos, with good results of subsequent thaw cycles, has been described (21).

The use of progesterone instead of HCG for luteal support is advised to reduce the risk for OHSS (19). However, support with progesterone during the luteal phase does not prevent OHSS. Nowadays, in our programme progesterone (200 mg vaginally twice daily; Progestan®; Organon) is routinely used when ≥15 follicles are aspirated during oocyte retrieval. Despite this, severe OHSS developed in 8 cases.

In an effort to prevent third space fluid accumulation, Asch et al. (36) treated high risk subjects for OHSS with albumin so as to increase the serum oncotic pressure, and possibly reverse the leakage of fluids from the intravascular space. The patients recieved 50 g of human albumin i.v. at the time of oocyte retrieval. Among 36 high risk patients no case of severe OHSS occurred. In a prospective placebo-controlled study, Shoham et al. (37) confirmed the possible role of the administration of albumin in the prevention of OHSS. The authors postulated that apart from the plasma expanding effect of albumin, the binding and transport properties of human albumin may play the main role in the prevention of severe OHSS by binding certain factor(s) that are possibly members of the renin-angiotensin cascade. However, absolute prevention of OHSS is not achieved with albumin administration. Two cases of OHSS occurring despite the administration of albumin have been described (38).

The key to prevention of OHSS is early identification of the patients at risk. To increase the predictability of OHSS, Delvigne et al. (39) developed a formula for pre-oocyte retrieval conditions in a multiple discriminant analysis. A prediction rate for the development of moderate or severe OHSS of 76.1% with a false-negative rate of 18.1% was found. The use of this formula calls for an intensive and expensive monitoring procedure and can not prevent OHSS in all cases.

Management of OHSS

Once OHSS develops, treatment should be aimed at prevention of serious complications, relief of symptoms, and shortening of hospital stay. The treatment of individual patients with OHSS varies according to its severity. Patients with moderate OHSS can be kept under surveillance as outpatients, cases of severe OHSS should be admitted. The patient should be carefully examined and the circulatory condition assessed. Vaginal examination is to be avoided because of the risk of injury to the ovaries. The ovaries should be screened by ultrasonography and the presence of ascites and pleural effusion noted. Basic laboratory investigations like full blood count, serum electrolytes and renal function tests should be carried out. The main line of treatment is to correct the circulatory volume and the electrolyte imbalance, which will improve the renal perfusion and prevent coagulation disorders. Colloidal plasma expanders such as dextran or albumin can be used to correct the hypovolemia. The hematocrit can serve as a guide during the treatment of the syndrome. Recording of the fluid balance is important, the urinary output must be carefully monitored. The use of heparin should be considered in severe OHSS in view of the risk of coagulopathy due to hemoconcentration. We used heparin 5000 IU twice daily for prophylactic reasons in 8 out of 18 cases of severe OHSS. Paracentesis is indicated when serious abdominal discomfort or breathing difficulties are caused by extensive ascites (17). Five of our patients

underwent abdominal paracentesis with marked relief of symptoms. To avoid injury to the enlarged ovaries, drainage of ascites may be performed via the transvaginal route under ultrasound guidance (20). A marked positive effect of paracentesis on the diuresis has been reported (16, 20). One of our patients had an extensive pleural effusion for which bilateral thoracal drainage was necessary. Remarkably, in this patient only a small amount of ascites was found. Hydrothorax has been described by others as the only extra-ovarian manifestation of OHSS (40).

As prostaglandins have been suggested to be important mediators of the increased vascular permeability in OHSS, prostaglandin synthetase inhibitors, like indomethacin, have been used for the treatment of the syndrome (4). However, Pride et al. (14), found no effect of the use of indomethacin on ascites formation in animal experiments. Balasch et al. (41) reported a case of severe OHSS complicated by prerenal oliguria and liver dysfunction due to indomethacin therapy. In view of the doubtful benefit of therapy with prostaglandin synthetase inhibitors and the possible side-effects, these drugs should not be used for the treatment of OHSS.

Secondary complications of OHSS

Although secondary complications of OHSS are rare, the severe form of OHSS should be considered a potentially life-threatening condition. Thromboembolism, acute pulmonary failure, hypovolemic shock, hepatocellular damage and renal failure (5, 16, 27, 41-43) are some of the complications that have been reported to occur in association with the syndrome. Mozes et al. (5) reported two cases of arterial thromboembolic complications. One patient died after a carotid arteriotomy and another one underwent a leg amputation. Cases of deep cerebral venous thrombosis as complication of severe OHSS have been described (43). Deep venous thrombosis in the absence of signs of hemoconcentration in a patient with a low level of antithrombin III was reported by Kaaja et al. (44). Hepatocellular damage may occur as a result of high oestrogen concentrations (41). Forman et al. (27) described a patient with severe OHSS who had a marked and prolonged elevation of liver enzymes, declining only after termination of the pregnancy which ended in a spontaneous abortion.

Among the cases of severe OHSS in our population no major complications occurred. In one case with moderate OHSS a ruptured ovarian cyst with intra-abdominal bleeding was diagnosed during a diagnostic laparoscopy for suspected adnexal torsion. Treatment remained conservative and the clinical course was uneventful.

Adnexal torsion

In the 2495 IVF cycles studied, adnexal torsion occurred in two cases. In one case a torsion and rupture of an ovary was diagnosed. After unwinding the ovary, suturing of the rupture, necessary because of bleeding, was not possible because the ovarian tissue was too friable. An adnex extirpation was carried out. In the other case an ovariectomy was carried out because the ovary was considerd too necrotic to justify a conservative approach.

Adnexal torsion is an infrequent but serious complication of ovarian stimulation and should be considered in every patient with complaints of abdominal pain and nausea during or after COH. Kemmann et al. (45) described 4 cases of adnexal torsion among 648 menotropin-induced pregnancies. Ovarian cysts are the main cause of adnexal torsion (46). Therefore the incidence of this complication can be expected to be higher among patients with OHSS. Mashiach et al. (47) found 15 cases of adnexal torsion in 201 cases of OHSS, 12 of whom were pregnant. The authors advised unwinding of the ovary regardless of its morphological appearance. This procedure was carried out in 11 of 12 patients. In 3 patients suture of the ruptured ovary was performed, is two cases ovarian cystectomy was necessary. Oelsner et al. (48) expanded on these data by reviewing the outcomes of 40 cases of adnexal torsion managed with detorsion only. In 26 cases laparotomy was carried out, the remaining 14 were managed with operative laparoscopy. The postoperative course was uneventful in all cases. In 35 of the 37 patients available for follow-up, a normal ovary with follicular development was noted on ultrasound examination. It appears that the sparing approach should be used in the management of adnexal torsion.

Complications of the transvaginal procedure

In the population studied, one serious visceral injury occurred in 2495 oocyte retrievals as reported elsewhere (49). This patient developed signs of appendicitis during the week after the IVF procedure. On the eighth day after oocyte retrieval a laparotomy was performed because of suspected peritonitis. A perforated appendix was removed. On histological examination the appendix showed several puncture holes. Intra-abdominal vascular injuries did not occur in our population.

Pelvic inflammatory disease (PID) was diagnosed in 6 patients after transvaginal oocyte retrieval, giving an incidence of 0.24% for this complication. The diagnosis was established by a rise in body temperature to >38° C and signs of pelvic peritonitis on physical examination, together with an elevated leucocyte count and erythrocyte sedimentation rate. All patients were

hospitalized for intravenous antibiotic treatment. In none of the cases a surgical approach was necessary. The mean duration of hospital admission was 13 days (range 4-21).

Oocyte retrieval for IVF was initially carried out by laparoscopy. The complications of this procedure were related to the anesthesia, pneumoperitoneum and visceral and vascular injuries caused by troicar insertion. The transvaginal ultrasound-guided follicle aspiration has replaced the laparoscopy nowadays and is used in the transport IVF programme since 1988. This procedure is carried out under local anaesthesia and is less time-consuming. Complications are infrequent but potentially serious. Pelvic visceral and vascular injuries have been reported (49, 50). Pelvic inflammatory disease is another potential hazard. Dicker et al. (51) reported 5 symptomatic injuries needing a surgical approach among 3656 ovum pick-ups: 2 patients with ruptured endometriotic cystic masses and 3 cases of intra-abdominal bleeding. A case of infected endometriotic cysts secondary to oocyte aspiration was described by Yaron et al. (52).

The risk of pelvic infection was initially seen as a disadvantage of the transvaginal approach. To reduce this risk, preventive antibiotics and vaginal desinfection have been proposed. (53-55). However, the efficacy of these measures has not been established. Børlum and Maigaard (56) reported 2 pelvic infections after 400 vaginal procedures using the semi-sterile technique and concluded that acceptable safety is achieved without the use of rigorous disinfection or preventive antibiotics. The low incidence of PID in our population supports this. Reports from large series show a low incidence of PID after transvaginal follicle aspiration. Ashkenazi et al. (57) found 28 cases among 4771 patients, and Bennett et al. (58) reported 18 cases after 2670 procedures, giving an incidence of 0.58% and 0.68% respectively. Pelvic infection after IVF treatment is not necessarily the result of transvaginal ovum pick-up since a case of PID as a result of embryo transfer in an oocyte donation cycle was reported by Sauer and Paulson (59).

Ectopic pregnancy

Thirteen out of the 624 pregnancies obtained in the IVF population studied were ectopics, giving an ectopic pregnancy rate of 2.1%. The incidence of ectopic pregnancy was analyzed according to indication for IVF. The results are shown in table 2. In one case an ectopic pregnancy was found in both fallopian tubes, in two cases an intra-uterine pregnancy was combined with an ectopic pregnancy.

The first pregnancy ever obtained after IVF was an ectopic pregnancy (60). The reported incidence of ectopic pregnancy after IVF-ET varies from 2 to 11% (61, 62). In view of the 1.2-1.4% incidence for all reported pregnancies (63), the risk for an ectopic pregnancy after IVF is

high and apparently related to the high incidence of tubal dysfunction in the IVF population. The incidence reported in multi-centric studies is approximately 5% (64, 65). The incidence for ectopic pregnancy of 2.1% in our population is low when compared with results of multi-centric studies. No statistically significant higher incidence was found in the group with tubal dysfunction. Conflicting data have been reported on the risk factors associated with ectopic pregnancies after IVF. Tubal dysfunction appears to be a risk factor (61, 65-69), and preventive measures prior to IVF treatment have been proposed. Steptoe et al. (70) advised occlusion of the utero-tubal junction on each side if diseased tubes are present but do not require removal. The same suggestion was made by other authors (71). However, Karande et al. (72) reported two interstitial pregnancies after IVF, one in a patient with a previous bilateral salpingectomy and the other in a patient who had a salpingectomy on the side of the ectopic pregnancy. Apparently, during embryo transfer embryos can be flushed into the fallopian tubes. When tubes are normal, the embryos return to the uterine cavity, simulating the physiological journey of the fertilized ovum. This transport may be thwarted when the tubes are dysfunctional.

Table 2. Rate of Ectopic Pregnancy According to Indication for IVF.

Indication	Pregnancies	Ectopic pregnancies
Tubal	297	8 (2.7) *
Non-tubal	327	5 (1.5) *†

* Values in parentheses are percentages of pregnancies.
† $P > 0.2$ (χ^2 test) when compared with ectopic pregnancy rate in tubal indications.

The embryo transfer technique has been suggested as a factor for the occurrence of ectopics. Knutzen et al. (73) injected 40 µl of radiopaque fluid in a mock embryo transfer and found flux into the fallopian tubes in 38% of patients. They advised transfer of embryos in a small amount (10-20 µl) of culture fluid in an attempt to prevent reflux. Yovich et al. (74) inserted the delivery catheter 55 mm only and concluded that this gives an embryo transfer to a standard midcavity position resulting in a lower ectopic pregnancy rate.

Evidence exists that ovarian stimulation is associated with an increased incidence of ectopic pregnancy (75). McBain et al. (76) found a high rate of ectopic pregnancy following ovulation induction in the absence of predisposing factors. The authors found an association between the occurrence of ectopics and an elevated urinary oestrogen excretion in the peri-ovulatory phase of induced ovulatory cycles, and concluded that high oestrogen levels influence tubal embryo

transport. The use of clomiphene citrate combined with HMG for COH has also been reported to be a risk factor (65, 68). Other authors could not confirm this (67, 69).

The incidence of combined intrauterine and extrauterine pregnancy is evidently higher after IVF than in spontaneous pregnancies. Rizk et al. (77) reported a frequency of 1% in clinical pregnancies after IVF. Marcus et al. (78) found 20 heterotopic pregnancies among 2650 clinical IVF pregnancies, giving a frequency of 0.75%. A heterotopic pregnancy can give diagnostic difficulties (79, 80), and should always be included in the differential diagnosis of symptomatic patients with an intrauterine pregnancy after IVF.

Conclusion

Our data and those reported in the literature show that IVF treatment, performed under good medical and laboratory practice conditions, carries an acceptable risk of complications. Strict prevention of OHSS is not possible. However, sharp attention to its symptoms and adequate treatment can lead to a less severe course of disease. Adnexal torsion occurs infrequently and ovariectomy is hardly ever indicated in these cases. Symptomatic visceral lesions and pelvic inflammatory disease as a result of follicle aspiration are rare. The risk for an ectopic pregnancy is higher when compared with the general population. Despite the low incidence of serious complications, counselling of patients on this aspect of IVF treatment is necessary.

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Chapter 8

A triplet pregnancy after in vitro fertilization is a procedure-related complication that should be prevented by replacement of two embryos only

Fertility and Sterility 1997;67:290-5

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Summary

To investigate whether the incidence and the outcome of triplet pregnancies after in vitro fertilization (IVF) treatment justify limitation of the number of embryos to be transferred to two only, the results of all triplet and higher-order pregnancies occurring in the transport IVF programme at the Zuiderziekenhuis were evaluated. The incidence of spontaneous reduction to a lower-order pregnancy, and the frequency of application of selective embryo reduction were studied. Triplet pregnancies, reaching at least 20 weeks' gestation were compared with 54 randomly chosen twin pregnancies from the same population, matched for maternal age and parity. The prevalence of triplet pregnancies after replacement of three embryos was evaluated.

Twenty-four out of 624 consecutive IVF pregnancies, occurring between januari 1989 and july 1994, were high-order pregnancies (23 triplets and one quadruplet). Three triplet pregnancies spontaneously reduced to twins. Selective embryo reduction was chosen as a therapeutic option by two patients with a triplet pregnancy and by the patient with a quadruplet pregnancy, giving an acceptance rate for this procedure of 12.5%. Comparison of triplets with twin pregnancies showed that more antenatal admissions of longer duration occurred during triplet pregnancies. A higher perinatal mortality in the triplet group caused 6 out of 18 patients with a triplet pregnancy to be confronted with at least one perinatal death. Triplets were born at a lower gestational age, had a lower birth weight, and a higher hospital admission rate of longer duration. When pregnancy occurred after replacement of three embryos, the risk of having a triplet pregnancy was 7.5%.

Conclusions: The poor obstetric outcome of triplet pregnancies in our population indicates that triplet pregnancies after IVF treatment have to be prevented. Selective embryo reduction is acceptable for few patients only, and can therefore not be seen as a solution. Replacement of three embryos results in a triplet pregnancy in an unacceptably high percentage. Replacement of two embryos only is the preferred method to prevent triplet pregnancies after IVF.

Introduction

Infertily treatment has a considerable influence on the incidence of triplet and higher-order pregnancies. About 70% of triplet pregnancies occurring nowadays result from ovulation induction or assisted reproductive technologies (ART) (1, 2). The high cost of maternal and neonatal care in high-order pregnancies has an effect on the use of health care resources (3) and will influence the public opinion about infertility treatment. The reported frequency of triplet and higher-order pregnancies occurring after IVF treatment is approximately 4-5% (4, 5).

Increased medical risks entailed by high-order pregnancies are well documented (6, 7). When resulting from an elective procedure like ART, these pregnancies should be considered iatrogenic procedure-related complications. Several authors stress the neccessity of avoidance of triplet and higher-order pregnancies in IVF programmes. Medical risks, psychological stress and associated costs of these pregnancies were reasons to suggest that more attention should be paid to approaches to infertility that reduce the likelihood of multiple gestation (3, 8, 9). However, the incidence of triplet pregnancies after IVF does not decrease (4, 5). Apparently, the embryo replacement policy has not been influenced by the publications mentioned above. For this there may be several explanations. In the first place, reported improvements in the outcome of multiple-gestation pregnancies (10, 11) may cause the perinatal morbidity and mortality in triplet and quadruplet pregnancies to be underestimated. Few authors report the obstetric outcome of their triplet and quadruplet IVF pregnancies. Evidence exists however, that high-order gestations in IVF populations are associated with a high perinatal mortality (12) and neonatal morbidity (6). A second reason for risking the occurrence of triplet and quadruplet pregnancies in IVF programmes may be the availability of techniques for selective embryo reduction in high-order pregnancies. Although improvements in the techniques for selective foeticide have been reported, the ethical aspects of the procedure are under debate and the risk of a total pregnancy loss is still considerable. This causes the unacceptability of selective embryo reduction for the majority of patients (7, 13). In the third place, the pursuit of high pregnancy rates can lead to replacement of

three or more embryos in IVF programmes. The 1990 results from the United States IVF-ET Registry show that 7% of the reporting clinics transfer on average more than five embryos per cycle (4). Azem et al. (14) report the replacement of six or more embryos in patients with repeated previous IVF failures. Although evidence exists that replacement of a higher number of embryos is associated with higher pregnancy and delivery rates (4), some authors found that replacement of two instead of three good embryos does not decrease the chance of a pregnancy significantly, but only decreases the multiple pregnancy rate (8, 9). Replacement of more than two embryos will inevitably lead to high-order pregnancies in some cases. The question is whether the possibly higher pregnancy rate outweighs the disadvantages and risks of high-order pregnancies.

The aim of this study is to investigate whether it is necessary to completely prevent the occurrence of triplet pregnancies in an IVF programme. This is done by evaluating the outcome of all triplet pregnancies in our programme. The acceptability of selective embryo reduction for patients in case a triplet or quadruplet pregnancy occurs is assessed. The results of IVF treatment after replacement of 2, 3 or 4 embryos is evaluated to investigate whether pregnancy rates after replacement of two embryos are acceptable. Finally, the risk of inducing a triplet pregnancy after replacement of three embryos is evaluated.

Materials and methods

During the period January 1989 to June 1994, 2495 oocyte retrievals for IVF treatment were carried out in the transport IVF programme at the Zuiderziekenhuis in Rotterdam. This programme, carried out in cooperation with the University Hospital Rotterdam, Dijkzigt, has been described elsewhere (15). Fresh embryo transfer took place in 2044 cycles, and in 1818 cases more than one embryo was available for replacement. The number of embryos replaced ranged from one to four, depending on the patient's age and the availability and quality of the embryos. Patients < 35 years of age were asked to consider replacement of not more than two embryos so as to avoid a triplet pregnancy. The final advice on the number of embryos to be replaced was given by the IVF laboratory staff. Embryo transfer was carried out on the 2nd to 5th day after follicle aspiration. The diagnosis triplet or quadruplet was made by transvaginal ultrasound examination at 7 to 9 weeks' pregnancy. The presence of embryos with heart activity confirmed the diagnosis, empty gestational sacs were not included. The incidence of spontaneous reduction to a lower-order pregnancy was studied. Patients with a triplet or quadruplet pregnancy were informed about the possibility of selective embryo reduction and were referred when they opted for this

procedure. Eighteen triplet pregnancies, reaching at least 20 weeks' gestation, were compared with 54 twin pregnancies randomly chosen from the same IVF population, matched for maternal age and parity. Triplet and twin pregnancies were compared for complications during pregnancy, perinatal mortality and neonatal morbidity. The results of the 1818 embryo transfers with replacement of two, three or four embryos were evaluated. The incidence of triplet pregnancy among pregnancies obtained after replacement of three embryos, and its relation to age was studied.

A clinical pregnancy was defined by a positive urinary pregnancy test 18 days after oocyte retrieval combined with the finding of a gestational sac two weeks later. An ongoing pregnancy was defined as an intact pregnancy at 12 weeks gestational age. Stillbirth was defined as intrauterine death after 20 weeks' gestation. For statistical analysis, Student's t-test was used for the difference between two means and the χ^2 test was used to compare the frequencies of observations. The Wilcoxon rank sum test was used where appropriate. $P < 0.05$ was taken as level of significance.

Results

During the study period 624 clinical pregnancies were obtained, 584 after fresh embryo transfer, 40 after replacement of cryopreserved embryos. Out of these pregnancies 476 were singletons, 124 twins, 23 triplets and one quadruplet. All 24 high-order pregnancies occurred after fresh embryo transfer, giving a high order pregnancy rate of 4%. Three patients, 2 with triplets and the one with quadruplets, opted for selective embryo reduction, giving an acceptance rate for this procedure of 12.5%. One of these triplets reduced spontaneously to twins before embryo reduction was carried out. Selective reduction of one triplet and the quadruplet pregnancy to twin pregnancies was performed without complications. Both patients delivered two healthy children at 36 and 37 weeks' gestation respectively. Spontaneous reduction to twins occurred in 2 additional triplet pregnancies. Out of the remaining 19 patients, one patient had an abortion at 17 weeks' gestation, resulting in a total pregnancy loss. The 18 triplet pregnancies reaching 20 weeks' gestation or more were compared with 54 twin pregnancies, randomly chosen from the same population, matched for maternal age and parity. Patient characteristics and complications during pregnancy are shown in table 1. Seventeen patients with triplet pregnancy were admitted antenatally. Routine admissions did not occur, cervix cerclage was not applied in any patient. Indications reported were: pregnancy induced hypertension (PIH), premature contractions,

premature rupture of membranes, maternal exhaustion and growth retardation or intrauterine death of one or more children. The admission rate and the number of antenatal hospital days for patients expecting triplets was significantly higher when compared with those expecting twins: $P < 0.0001$ and $P < 0.01$ respectively. A higher incidence was found for PIH in the triplet pregnancy group. No statistically significant differences were found for the incidence of premature rupture of membranes and the use of intravenous tocolitics for premature contractions in the two groups. All triplets were born before 37 weeks' gestation was reached, 44% of the twins were born prematurely.

Table 1. Complications in Triplet and Twin Pregnancies Completing 20 Gestational Weeks

	Triplets (n=18)	Twins (n=54)	P value
Maternal age (y) *	32.0 ± 3.2	31.7 ± 3.1	NS †
Primigravid patients ‡	17 (94)	51 (94)	NS
Antenatal hospitalization	17 (94)	19 (35)	< 0.0001
Hospital days *	27.8 ± 20.1	12.4 ± 11.5	< 0.01
Pregnancy-induced hypertension (PIH)	6 (33)	3 (6)	< 0.01
Premature contractions + i.v. tocolytics	7 (39)	10 (19)	NS
Premature rupture of membranes	1 (6)	4 (7)	NS
Premature delivery §	18 (100)	24 (44)	< 0.001
<30 weeks	2	1	
31-34 weeks	13	7	
35-36 weeks	3	16	

* Values are means ± SD.

† NS, not significant.

‡ Values in parentheses are percentages.

§ Delivery before completion of 37 gestational weeks.

The vital statistics of the pregnancy outcome of the triplet and twin pregnancies are shown in table 2. Nine intrauterine deaths, discovered between 22 and 33 weeks' gestation, occurred in 6 pregnancies in the triplet group. In one case all 3 children died at 22 weeks' gestation on 3 consecutive days during admission for premature contractions. For all cases no explanation could be found after birth. In both groups 2 early neonatal deaths occurred, all due to complications after an early premature delivery. The difference for perinatal mortality between the two groups is highly significant: $P < 0.0001$.

Table 2. Vital Statistics of Pregnancy Outcome of the Triplet Pregnancies Compared with Twin Pregnancies.

	Triplets (n = 54)	Twins (n = 108)
Stillbirt	9	1
Early neonatal death (≤ 7 days)	2	2
Late neonatal death (8-28 days)	0	0
Perinatal deaths *†	11 (20)	3 (3)

* Values in parentheses are percentages of births.

† $P < 0.0001$ for comparison between groups.

The neonatal outcome of the live-born children after triplet or twin pregnancies is shown in table 3. A statistically significant difference was found for gestational age and birth weight. A higher percentage of the triplets were admitted; 87% compared with 53% of twins. The duration of hospital stay for admitted children was significantly longer for triplets compared with twins.

Table 3. Outcome of Live-Born Infants in Triplet and Twin Pregnancies.

	Triplets (n=45)	Twins (n=107)	*P* value
Gestational age, median days (range)	237 (193-255)	259 (163-283)	< 0.0001
Birth weight (g) *	1830 (720-2670)	2470 (550-3700)	< 0.0001
< 1500 g †	10 (22)	5 (5)	
1500-2499 g †	31 (69)	51 (47.5)	
≥ 2500 g †	4 (9)	51 (47.5)	
Hospital admission †	39 (87)	57 (53)	< 0.001
Hospital stay (d) *	35 (5-59)	18 (2-64)	< 0.0001
Neonatal intensive care unit (NICU) †	4 (9)	2 (4)	< 0.05
Sepsis †	3 (7)	1 (1)	< 0.05
Neonatal mortality †	2 (4)	2 (2)	NS ‡

* Values are medians with range in parentheses. ‡ NS, not significant.

† Values in parentheses are percentages.

In the triplet group, admission to the neonatal intensive care unit (NICU) and neonatal sepsis occurred more frequent. Convulsions were not seen in the triplet group and occurred in one child in the twin group. No cases of necrotizing enterocolitis or intraventricular hemorrhage occurred in either group. Two infants in the triplet group, born at a gestational age of 193 days, had a severe respiratory distress syndrome and died 6 days after birth. The 2 neonatal deaths in the twin group occurred in twins born at the gestational age of 163 days. Both children died immediately after birth and were not admitted to the NICU. The resulting neonatal mortality in the 2 groups is not statistically significant.

Table 4. IVF Results in Relation to Number of Embryos Transferred.

	ET n = 2	ET n = 3	ET n = 4
Number of cycles	732	777	309
Clinical pregnancies *	220 (30 [27-33])	241 (31 [28-34])	99 (32 [26-37])
Ongoing pregnancies *	176 (24 [21-27])	193 (25 [22-28])	80 (26 [21-31])
Multiple pregnancies †	49 (22 [16-28])	73 (30 [23-35])	28 (28 [18-37])

* Values in parentheses are percentages per ET with 95% confidence interval in brackets.
† Values in parentheses are percentages per clinical pregnancy with 95% confidence interval in brackets.

The results of 1818 fresh embryo transfers with 2 or more embryos available, are shown in table 4, and table 5 shows the results of IVF treatment with replacement of 3 embryos in three different age groups. The incidence of triplet pregnancy among pregnancies obtained after replacement of 3 embryos was 7.5%. In the age group ≥ 35 years 4 triplet pregnancies occurred among 68 clinical pregnancies. Three of these pregnancies were ongoing, giving a triplet pregnancy rate of 6% for ongoing pregnancies.

Discussion

The incidence of IVF triplets can be expressed as a percentage of all IVF births, or as a percentage of births after transfer of three embryos, in our study 3.8% and 7.5% respectively. In the survey of data presented by the SART (5) the triplet incidence is 5.4% of all deliveries. It is likely that the

triplet incidence in pregnancies resulting from the transfer of three embryos will be considerably higher. Spontaneous reduction appears to play a minor role in the natural course of high- order pregnancies in the population studied. Spontaneous embryo reduction to a twin pregnancy occurred in 3 out of 23 triplet pregnancies only, giving an incidence of 13% per pregnancy. The incidence of spontaneous embryo reduction in multiple pregnancies reported in the literature varies and seems to depend on the inclusion criteria used in the various studies. Manzur et al. (16) counted gestational sacs measuring ≥ 5 mm in diameter on days 21 to 28 after embryo transfer, and found an incidence of vanishing embryos in triplet pregnancies of approximately 50%. Other authors diagnosed a spontaneous reduction only after disappearance of formerly viewed fetal cardiac motion and found an incidence of vanishing triplet of 16% per pregnancy (12) or a 5% rate of spontaneous fetal demise for a specific embryo in multiple gestation (17). In this study we used the same criteria and found spontaneous fetal demise in 3 out of 73 initially viable embryos, giving a rate of 4% per embryo.

Table 5. Age of the Patient and Replacement of Three Embryos.

Age category (years)	Total	< 30	30-34	≥ 35	P value *
Embryo transfers no.	777	210	333	234	
Clinical pregnancies †	241 (31.0)	63 (30.0)	110 (33.0)	68 (29.1)	0.80
Ongoing pregnancies †	193 (24.8)	52 (24.8)	91 (27.3)	50 (21.4)	0.38
Multiple pregnancies ‡	73 (30.3)	22 (34.9)	34 (30.9)	17 (25.0)	0.07
Twins ‡	55 (22.8)	17 (27.0)	25 (22.7)	13 (19.1)	0.30
Triplets ‡	18 (7.5)	5 (7.9)	9 (8.2)	4 (5.9)	0.62

* χ^2 test for trend between the three age groups.

† Values in parentheses are percentages per ET.

‡ Values in parentheses are percentages per clinical pregnancy.

Selective embryo reduction as a therapeutic option for high-rank pregnancies is under debate. The different terms used for the procedure: selective termination, selective foeticide or selective continuation of pregnancy seem to indicate differences in appreciation. Ethical objections and medical risks of the procedure can not be ignored. Evans et al. (18) argued that selective reduction in high-rank pregnancies is ethically justified because it meets the criterion of least harm and most potential good. Though perinatal morbidity and mortality are likely to be improved when pregnancies with four or more fetuses are reduced to smaller numbers (19), the

advantages of reducing the fetal number in triplet pregnancies are less apparent (7, 20). Reduction of triplets to twins gives a fetal loss of 33% by definition. The risk of total pregnancy loss after selective embryo reduction is considerable and appears to vary with the method applied. The transabdominal and transcervical selective termination is associated with a total pregnancy loss of approximately 12%, compared with 5.3% for the early transvaginal embryo aspiration (21). Comparative studies of the outcome of triplet pregnancies managed expectantly or by selective embryo reduction to twins have been published. Bollen et al. (12) reported that the pregnancy outcome of triplets is improved after selective reduction. A prospective study was carried out by Lipitz et al. (7). The authors reported a satisfactory outcome of pregnancy, defined as the discharge home of at least one infant, in 88.2% of the group with reduction to twins and in 74.5% of the triplets managed expectantly. However, this difference was not statistically significant.

We informed our patients about the possibility of selective embryo reduction and about the possible risks and benefits. Only 3 out of 24 patients with a triplet or quadruplet pregnancy opted for selective embryo reduction, giving a acceptance rate for this procedure of 12.5%. A report from Sweden about multiple fetal reduction showed a comparable low acceptance rate of 14% in triplet and higher-order pregnancies (13). Apparently, the emotional aspects and the procedure-related risks of selective embryo reduction make the procedure inacceptable for the majority of patients. Therefore, selective embryo reduction can not be seen as the solution when high-order pregnancies occur after ART.

In our population the antenatal admission rate and the duration of admission of patients expecting triplets was significantly higher when compared with those expecting twins. All patients with a triplet pregnancy delivered before the completion of 37 gestational weeks. Sassoon et al. (22) reported preterm labor as the only antenatal complication occurring significantly more often in triplet pregnancies compared with twins.

The vital statistics of triplets and twins show a high number of stillbirths in the triplet group. Six out of 18 patients with a triplet pregnancy were confronted with at least one perinatal death. The difference with the control twin population is highly significant. Although the neonatal mortality rate is low when compared with other reports, the resulting perinatal mortality is high. This finding confirms a report by Bollen et al. (12). Other authors reported better outcomes of triplet pregnancies. The perinatal and neonatal mortality rates of 78 triplet pregnancies reported by Lipitz et al. (11) were 93/1000 and 51/1000 respectively. Newman et al. (10) found a perinatal mortality rate of 6.6% in 198 triplet pregnancies. Population differences may be an explanation for differences in obstetric outcome. Only 9% of the patients of Lipitz et al. (11), and 7% of the patients of Newman et al. (10), became pregnant after IVF. Under-reporting in literature of less

favourable results can be another explanation for finding a higher perinatal mortality rate in our population when compared with other studies.

The median for gestational age and birthweight of live-born triplets is significantly lower when compared with twins. In our study, gestational age and birthweight of twins and triplets are comparable with data reported by other authors (7, 22). The higher admission rate and longer duration of admission of triplets appears to be related to prematurity. Serious complications during the early neonatal period occurred infrequently in both groups. The neonatal mortality in the two groups did not differ significantly. Comparable findings were reported by Sassoon et al. (22). Other authors (16) report a high neonatal morbidity requiring complex and expensive medical care and prolonged hospitalization.

Considering the low acceptance rate of selective embryo reduction and the high perinatal mortality in the triplet population studied, it appears that prevention, by replacing a maximum number of two embryos, is the approach to be preferred to solve the high-order pregnancy problem in ART. The objection to this approach is a possible lowering of the pregnancy rate. Staessen et al. (8) showed that the pregnancy rate is not significantly affected by replacing two embryos only in cycles with a good IVF prognosis. A good prognosis was defined by the criteria: first attempt for IVF, age below 37 years, and good embryo development. Tasdemir et al. (9) reported that transfer of two embryos only does not affect the pregnancy rate as long as one good quality embryo is available. In several other studies (23, 24) it was shown that elective transfer of 2 embryos can lead to a high pregnancy rate.

In the present study it appeared that 6% of the ongoing pregnancies obtained after replacement of three embryos in patients aged 35 years and older were triplets. This risk of a triplet pregnancy in older patients is considered unacceptably high and indicates that there is no justification for a more liberal replacement policy in higher age groups. Therefore, at present, no more than two embryos are replaced in any patient in the IVF programme in Rotterdam. Limitation of the number of transferred embryos increases the need for optimal embryo selection at transfer. Prolonged embryo culture seems to be the method of choice to achieve this (25). With replacement of two embryos, high costs of prolonged hospitalization and complex and expensive medical care needed in high-order pregnancies after ART are avoided. In The Netherlands, where health insurances pay the costs of three IVF attemps, resources saved in this way could be used to offer patients a fourth IVF procedure if needed. This would compensate for the possibly lower pregnancy rate caused by replacement of two embryos only.

In conclusion, spontaneous embryo reduction plays a minor role in the natural course of high-order pregnancies, and selective embryo reduction is not considered an acceptable therapeutic option by the majority of patients with triplet pregnancies. The perinatal mortality in the triplet

population studied is high when compared with twin pregnancies. The only good solution for the problem of the occurrence of high-order pregnancies in ART is strict limitation of the number of embryos to be replaced to two.

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3.
The expression of success rates

Chapter 9

Cumulative pregnancy rate and drop-out of patients in IVF

Submitted for publication

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Summary

The validity of cumulative pregnancy rates is tested by the determination of possible influences of selective drop-out of patients with a poor treatment prognosis. A cohort of 1211 patients who had a first IVF cycle was followed, and the cumulative pregnancy rate (CPR) after 3 IVF cycles was assessed. Cycles of patients who discontinued treatment after failed IVF, and of those who did not achieve a pregnancy but proceeded to a subsequent cycle were compared for occurrence of prognosticators of poor treatment outcome: fertilization rate, oocyte yield ≤ 2, and replacement of < 2 embryos. The CPR after 3 cycles was 55.1%. No differences were found between patients who continued treatment and those who dropped out.

Conclusion: Selective drop-out of patients with a poor treatment prognosis was not found. Therefore, the CPR of 55.1% after 3 IVF cycles gives a reliable indication of the chance of occurrence of a pregnancy for a randomly chosen patient in the population studied.

Introduction

Patients should be provided with adequate information about their chances of pregnancy before they start IVF treatment. The estimation of the likelihood of pregnancy for couples is often based on the pregnancy rate per cycle obtained in a programme. However, several methods can be used to express pregnancy rates (PR) in IVF programmes. The definition of clinical or ongoing pregnancy can differ between programmes, and PR can be calculated per started cycle, per oocyte retrieval, or per embryo transfer. When informing patients about success rates, a possible reduction in PR in successive cycles should be considered. This phenomenon has been reported

by several authors (1, 2), and is explained as the result of heterogeneity of the patient population in terms of fecundity rate. For a randomly chosen patient each unsuccessful cycle constitutes evidence in favor of low fertility potential.

The success rate in an IVF programme can also be expressed as a cumulative pregnancy rate (CPR). When CPRs are used, the danger exists that unrealistic high success rates will be found. Not all patients continue IVF treatment until pregnancy or for a fixed number of attempts. Therefore, when CPRs are used, assumptions are made about the probability of occurrence of a pregnancy for those who discontinue treatment. Most authors who report on CPRs assume that patients who leave an IVF programme had the same probability of pregnancy as those who continued (2, 3). For example, Guzick et al. (3) reported a CPR of 100% after 9 IVF cycles in the subgroup with tubal infertility in their population, while out of the 394 patients entering the study in this subgroup only 105 achieved a pregnancy. It is very likely that those who stopped treatment because of poor results in a preceding IVF attempt in terms of oocyte yield, fertilization rate, or number of embryos available for replacement, would have had a lower chance of pregnancy than those who continued. For this reason Stolwijk et al. (4) advised to consider the reason for cessation when calculating the CPR.

To obtain CPRs for the transport IVF programme at the Zuiderziekenhuis, Rotterdam, a retrospective cohort study was carried out. To test the validity of the rates obtained, patients who left the programme without a pregnancy (drop-outs) were compared with those who continued for the occurrence of prognosticators of poor treatment outcome in the preceeding failed IVF cycle.

Materials and methods

Patients who had their first IVF attempt during the period January 1989 - June 1994 were included in the study. The consecutive IVF cycles of these patients, until the occurrence of a clinical pregnancy, with a maximum of 3 cycles, were used to calculate the CPR after 3 IVF cycles. The patients who did not obtain a clinical pregnancy in the first or second cycle were divided in 2 groups: those who left the programme (drop-outs), and those who proceeded to a next attempt. These two groups were compared for age, and the preceding IVF cycles were compared for fertilization rate, and for the occurrence of prognosticators of poor treatment outcome: oocyte yield ≤ 2, and replacement of < 2 embryos.

A clinical pregnancy was defined by a positive urinary test 18 days after oocyte retrieval combined with the finding of a gestational sac 2 weeks later. The results of cryopreservation-thaw

cycles were included in this analysis. For statistical analysis χ^2 test or Students' t-test was used where appropriate. $P < 0.05$ was taken as level of significance.

Results

During the study period 1211 patients entered the IVF programme. The results of this cohort are shown in table 1. A statistically significant decrease in pregnancy rate was found with increasing cycle number.

Table 1. IVF Results of the First Three Cycles.

Attempt	Patients	PR*†	CPR	Drop-outs
1	1211	26.6	26.6	263
2	624	24.5	44.6	193
3	280	18.9	55.1	

*Pregnancy rate; percentage clinical pregnancies per oocyte retrieval.
† $P < 0.01$ for differences between attempt 1, 2 and 3 (χ^2 for trend)

Characteristics of the first IVF cycle of drop-outs and those who continued are shown in table 2.

Table 2 . Characteristics of the First Cycles of Drop-outs versus Those who Continued.

	Drop-outs	Continued	P value
Number of patients	263	624	
Age	32.4 ± 4.6	32.3 ± 4.4	NS *
Fertilization rate	43 %	45 %	NS
Oocytes ≤ 2	11.4 %	12.8	NS
ET < 2	37.3 %	34.2 %	NS

* NS, not significant.

Characteristics of the second IVF cycle of drop-outs and those who continued are shown in table 3.

Table 3 Characteristics of the Second Cycles of Drop-outs versus Those who Continued.

	Drop-outs	Continued	P value
Number of patients	193	280	
Age	32.4 ± 4.3	32.7 ± 4.4	NS *
Fertilization rate	46 %	45 %	NS
Oocytes ≤ 2	10.8 %	!0.8 %	NS
ET < 2	39.5 %	35.9 %	NS

* NS, not significant.

Discussion

When patients are informed about their chances of pregnancy in IVF treatment, the shortcomings of the different methods used to express the success rates of IVF programmes should be kept in mind. When the success rate in an IVF programme is expressed as PR per IVF cycle, a possible reduction in PR in successive cycles should be considered. Hershlag et al. (1) reported that the probability of achieving a pregnancy declines as the number of unsuccessful cycles increases. Using a mathematical model, the authors estimated that 37% of couples will not conceive with IVF therapy despite multiple attempts. They therefore questioned the justification of continuing IVF treatment beyond a threshold number of cycles. In contrast, Guzick et al. (3) found that persistance in IVF can lead to a successful pregnancy for a large proportion of couples. The authors reported an approximately constant pregnancy rate of about 15% over repeated cycles. The predicted cumulative pregnancy rate after 9 and 12 cycles were 75% and 84% respectively, and, consequently, a 98% cure rate was predicted if multiple cycles were pursued. These contradictions in reported results of different programmes may be explained as the result of selection bias. In the first place, different selection criteria will cause different patient populations at intake. Secondly, some programmes may encourage patients to discontinue treatment after poor performance in the previous IVF cycle, whereas other programmes might encourage these patients to proceed to a next attempt.

Haan et al. (5) used pre-treatment patient characteristics to make a comparison of the prognosis for success between 'continuers' and 'quitters', and found no over-representation of patients with a poor prognosis in the group of drop-outs. However, disappointing results in terms

of low oocyte yield (≤ 2), low fertilization rate, and replacement of a suboptimal number (< 2) of embryos in a previous cycle might be more indicative of poor treatment prognosis than pretreatment characteristics (6). Selective patient drop-out, and consequently selection bias, can be expected when the frequency of occurrence of these prognosticators of poor treatment outcome are higher in patients who discontinue treatment after a failed IVF attempt.

In The Netherlands the first 3 IVF attempts are paid for by health insurances. Therefore, for patients eligible for IVF treatment in our population, the CPR after 3 IVF cycles is the most useful information before the treatment is started. In this study the CPR after 3 cycles was calculated, and previous cycles of those who continued or dropped out after failed first and second IVF attempts were compared for prognosticators of poor treatment outcome. No statistically significant differences were found between the 2 groups. Despite this, the PR declined significantly as the cycle number increased, confirming reports by others about the phenomenon of reduction in PR in successive IVF cycles (1, 2).

In conclusion: selective drop-out of patients with a poor treatment prognosis was not found. Therefore, the CPR of 55.1% after 3 IVF cycles found in this study gives a reliable indication of the chance of occurrence of a pregnancy for a randomly chosen patient in our population. Pregnancy rates decline with increasing cycle number.

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Summary

In the **general introduction** contentious issues in assisted reproductive technology (ART) are mentioned, and the objectives of this thesis are explained. The main objectives are: to determine the efficacy of a decentralized IVF progamme with transport and satellite clinics and to further develop criteria for acceptance of patients for IVF treatment. Furthermore, to develop a safer embryo replacement strategy, and, where possible, to touch upon controversial issues in ART.

The thesis is divided in two parts. In part one the logistical and organizational aspects of the IVF programme at the Zuiderziekenhuis, Rotterdam, are studied, and the feasibility of a simplified protocol for monitoring of patients during controlled ovarian hyperstimulation (COH) is assessed. Clinical aspects of IVF treatment are studied in part two. This part is divided into three separate sections dealing with: aspects of patient selection for IVF, the occurrence of procedure-related complications, and the expression of success rates in IVF pogrammes.

Chapter 1 is an evaluation of the results obtained in a decentralized IVF programme with a transport IVF clinic, satellite clinics and a central University IVF laboratory. The treatment results obtained in the decentralized programme were compared with those obtained in the university IVF centre using the same laboratory facilities. Transportation of oocytes after follicle aspiration to a central laboratory, and monitoring of COH by physicians in satellite clinics appeared to have no adverse effects on the outcome of IVF treatment. In a decentralized programme laboratory facilities can be utilized more efficiently. Moreover, a decentralized programme is more accessible for patients in peripheral locations.

Chapter 2 is a prospective study carried out to assess the feasibility of minimal monitoring during COH. In the transport IVF programme studied, monitoring of COH is carried out without estradiol assay but with ultrasound measurements only. The monitoring procedure in the transport clinic was minimized, while the monitoring protocol used in the satellite clinics, where physicians monitored their own patients, remained unchanged. In a prospective study minimal monitoring appeared to have no adverse effects on the treatment outcome. Sixty-two percent of cycles at the transport clinic were monitored with one ultrasound measurement only. There appears to be no need for extensive monitoring during COH for IVF.

Chapter 3 is a review of the literature about the logistical advantages of gonadotrophin-releasing hormone agonists (GnRH-a) in IVF. Moreover, the experience with the use of GnRH-a in the

transport IVF programme is reported. During routine use of GnRH-a the short flare-up protocol and the long protocol give comparable clinical results. Both protocols are useful for treatment planning and offer important logistical possibilities. In terms of cost, time and inconvenience for patients, the short flare-up protocol seems to be the most appropriate regimen for routine use. The intranasal administration of GnRH-a appears to be equally efficacious when compared with the subcutaneous route.

Chapter 4 reviews the literature about the relation between age and outcome of IVF treatment. The patient's age, although an indicator of fecundity, should not be the main parameter for acceptance of patients for IVF treatment. Results of prestimulation tests like the determination of basal follicle-stimulating hormone level and the clomiphene citrate stimulation test are more useful for counselling of patients before treatment is started.

Several proposals have been made to improve the treatment outcome of IVF in older women. The administration of higher dosages of gonadotrophins during COH and GnRH-a in the flare-up regimen has been proposed for "poor responders". Assisted hatching of embryos with a thick zona pellucida has been reported to improve the embryo implantation rate. Replacement of higher numbers of embryos is seen as an option by some authors. Oocyte donation, although controversial in postmenopausal women, is a good alternative for older patients who fail to concieve with their own oocytes.

In **chapter 5** a retrospective study, carried out to determine whether age or response to COH is a better predictor of IVF outcome in women ≥ 40 years of age, is described. The frequency of occurrence of a poor ovarian response to COH increased significantly with rising age. Analysis of all cycles showed a significant decrease in clinical and ongoing pregnancy rates for women ≥ 40. Analysis of cycles with a good ovarian response showed no statistically significant difference for these parameters between women aged ≥ 40 and those younger. A logistic regression analysis on pregnancy showed that ovarian response contributed more to the prediction of pregnancy than age. A strict age limit for acceptance of patients for IVF treatment should therefore not be used. Instead, the response to COH can be used to select patients likely to have a successful treatment outcome.

Chapter 6 deals with the treatment prognosis after poor fertilization in the first IVF cycle. Total fertilization failure and poor fertilization were defined. The relation between fertilization rate and the number of motile sperm cells/mL semen was evaluated. Three patient groups with different semen quality in the first IVF cycle were defined. In all groups poor fertilization appeared to have

a high chance of recurrence in a second IVF cycle. In the population studied the use of intracytoplasmic sperm injection should be considered after poor fertilization in the first IVF cycle, especially in cases with a small oocyte yield.

Chapter 7 is a review of the literature about the occurrence of procedure-related clinical complications of IVF treatment. In this chapter the clinical complications which occurred in 2495 consecutive cycles are reported. Four different complications leading to hospital admission were identified: the ovarian hyperstimulation sydrome, adnexal torsion, pelvic inflammatory disease, and ectopic pregnancy. A total number of 51 patients with complications was admitted. It is necessary to counsel patients for the occurrence of serious procedure-related complications before IVF treatment is started.

In **chapter 8** the outcome of all high-order pregnancies obtained in 2495 consecutive IVF cycles is described. The occurrence of spontaneous reduction to a lower-order pregnancy, and the acceptability of selective embryo reduction were studied. Twenty-four out of 624 consecutive pregnancies were high-order pregnancies (23 triplets and one quadruplet). Eighteen triplet pregnancies reached at least 20 weeks' gestation and were compared with 54 randomly chosen twin pregnancies from the same population, matched for maternal age and parity. The results of IVF treatment after replacement of 2, 3 or 4 embryos were evaluated, and the chance of occurrence of a triplet pregnancy after replacement of 3 embryos was assessed. Selective embryo reduction was chosen as a treatment option by two patients with a triplet pregnancy and by the patient with quadruplets, giving an acceptance rate for this procedure of 12.5%. Three triplet pregnancies reduced spontaneously to twins. A high number of perinatal deaths (11/54) was found in the triplet group, causing 6 out of 18 couples with a triplet pregnancy to be confronted with the loss of at least one child. When compared with twins, triplets were born at a lower gestational age, had a lower birth weight, and a higher hospital admission rate of longer duration. When a pregnancy occurred after replacement of three embryos, the risk of having a triplet pregnancy was 7.5%. Replacement of two embryos only gave acceptable treatment results and is the method chosen in the IVF programme in Rotterdam to prevent triplet pregnancies.

In **chapter 9** the various methods to express the success rates of an IVF programme are discussed. The cumulative pregnancy rate (CPR) after three attempts in the IVF programme studied was calculated, and the validity of this CPR was tested by assessing a possible influence of drop-out of patients with a poor treatment prognosis. Cycles of patients who discontinued treatment after failed IVF and of those who did not obtain a pregnancy but proceeded to a subsequent cycle were

compared for fertilization rate and for occurrence of prognosticators of poor treatment outcome: oocyte yield ≤ 2 and replacement of < 2 embryos. The CPR after 3 cycles was 55.1%. No differences were found between patients who continued treatment and those who dropped out. Selective drop-out of patients with a poor treatment outcome was not found. Therefore, the CPR of 55.1% after 3 IVF cycles gives a reliable indication of the chance of occurrence of a pregnancy for a randomly chosen patient in the population studied. Pregnancy rates decline with increasing cycle number.

Conclusions

- Decentralization of IVF treatment with transport and satellite clinics has no adverse effects on the treatment outcome and offers several advantages to the patients.
- In most cases the monitoring of controlled ovarian hyperstimulation for IVF can be limited to one ultrasound measurement only.
- An age limit of 40 years for acceptance of patients for IVF is not justified.
- Poor fertilization has a high chance of recurrence in a second IVF cycle, irrespective of the number of motile sperm cells/mL semen in the first cycle.
- In approximately 2% of cases IVF treatment gives procedure-related clinical complications leading to hospital admission.
- Triplet pregnancies after IVF should be prevented by replacement of two embryos only.
- Cumulative pregnancy rates should be validated by analysis of the treatment performance of those who discontinued treatment.

Samenvatting

In de **inleiding** worden controversiële onderwerpen op het gebied van de geassisteerde voortplantingstechnieken genoemd en worden de doelstellingen van dit proefschrift vermeld. De doelstellingen zijn: Het vaststellen van de uitvoerbaarheid en doeltreffendheid van een gedecentraliseerd IVF programma met transport- en satellietklinieken. Het verder ontwikkelen van kriteria voor het toelaten van patiënten voor IVF behandeling en het ontwikkelen van een veiliger beleid bij het terugplaatsen van embryo's. Verder zullen controversiële onderwerpen op het gebied van IVF besproken worden.

Dit proefschrift bestaat uit twee delen. In deel 1 worden de logistieke en organisatorische aspekten van het IVF programma van het Zuiderziekenhuis en de uitvoerbaarheid van een vereenvoudigd protocol voor monitoren tijdens gecontroleerde ovariële hyperstimulatie bestudeerd. Klinische aspecten van de IVF behandeling worden bestudeerd in deel 2. Dit deel is in drie secties onderverdeeld waarin behandeld worden: aspekten van selectie van patienten voor IVF, het vóórkomen van complicaties ten gevolge van de behandeling, en de wijze waarop succespercentages in IVF programma's worden weergegeven.

Hoofdstuk 1 is een evaluatie van de resultaten verkregen in een gedecentraliseerd IVF programma met een transport IVF-kliniek, satellietklinieken en een centraal universitair IVF laboratorium. De verkregen behandelingsresultaten van het gedecentraliseerde programma werden vergeleken met de resultaten verkregen in de universitaire IVF-kliniek. Beide programma's werken samen met hetzelfde IVF-laboratorium. Transport van eicellen na eicelpunctie naar het centrale IVF-laboratorium gevolgd door embryoterugplaatsing in de universitaire IVF-klinick bleek geen nadelige effecten op de uitkomst van de IVF-behandeling te hebben. In een gedecentraliseerd programma kunnen laboratoriumfaciliteiten efficiënter benut worden. Bovendien is een gedecentraliseerd programma toeganke-lijker voor patienten die verder van IVF klinieken verwijderd wonen.

Hoofdstuk 2 is een prospectieve studie naar de toepasbaarheid een tot een minimum beperkte monitoring procedure tijdens de gecontroleerde ovariële hyperstimulatie. In het transport IVF-programma werd de ovariële hyperstimulatie vervolgd door middel van echoscopisch onderzoek zonder bepalingen van het oestradiol in het serum. Het protocol voor het vervolgen van de ovariële reactie werd in de transport klinick veranderd met het doel het aantal echoscopiën tot een minimum terug te brengen. Het protocol voor de satellietklinieken, waar artsen de eigen patiënten

vervolgden, bleef ongewijzigd. In een prospectieve vergelijkende studie bleek het nieuwe protocol geen nadelige effecten op de uitkomst van de IVF-behandeling te hebben. Bij 62% van de cycli die in de transport kliniek vervolgd werden bleek één echoscopie afdoende. Het intensief vervolgen van de ovariële hyperstimulatie voor IVF blijkt derhalve niet noodzakelijk te zijn.

Hoofdstuk 3 is een overzicht van de literatuur over de logistieke voordelen van gonadotropine-releasing hormoon agonisten (GnRH-a) bij IVF, en een evaluatie van het gebruik van GnRH-a in het transport IVF-programma. Bij routine gebruik van GnRH-a geven het korte flare-up protocol en het lange protocol vergelijkbare klinische resultaten. Beide protocollen kunnen gebruikt worden voor planning van de behandeling en bieden belangrijke logistieke mogelijkheden. Het korte flare-up protocol lijkt het meest aangewezen protocol met het oog op kosten, tijd en ongemak voor de patient. De intra-nasale toediening van GnRH-a blijkt even goed toepasbaar als de subcutane toediening.

Hoofdstuk 4 is een overzicht van de literatuur over de relatie tussen leeftijd en uitkomst van IVF-behandeling. Hoewel de leeftijd van de patiënt een rol speelt bij de vruchtbaarheid, dient leeftijd niet de belangrijkste parameter te zijn bij het accepteren van patiënten voor IVF. Bepaling van het basaal follikel-stimulerend hormoon en de clomifeen citraat stimulatie test zijn beter bruikbaar voor het voorlichten van patiënten voor aanvang van de behandeling.

Verschillende methoden zijn voorgesteld om de resultaten van IVF bij oudere patiënten te verbeteren. De toediening van hoge doseringen gonadotrofines en het gebruik van GnRH-a in het flare-up protocol zijn voorgesteld voor patiënten met een slechte ovariële reactie tijdens gecontroleerde ovariële hyperstimulatie. De terugplaatsing van hogere aantallen embryo's wordt door enkele auteurs als een behandelingsmogelijkheid gezien. Eiceldonatie, hoewel controversieel bij postmenopausale vrouwen , is een goed alternatief voor oudere vrouwen met slechte behandelingsresultaten.

Hoofdstuk 5 is een retrospectieve studie uitgevoerd om te bepalen of de leeftijd of de ovariële reactie op hyperstimulatie de betere voorspeller is van de uitkomst van IVF-behandeling bij vrouwen ouder dan 40 jaar. Een slechte ovariële reactie op gecontroleerde hyperstimulatie wordt significant vaker gezien bij oudere patiënten. Bij een analyse van alle uitgevoerde IVF-behandelingen wordt een significante daling van het percentage klinische en doorgaande zwangerschappen per behandeling gezien bij patiënten ouder dan 40 jaar. Echter, bij analyse van behandelingen waarbij sprake was van een goede ovariële reactie bleek er voor deze parameters geen statistisch significant verschil te bestaan tussen patiënten ouder en jonger dan 40 jaar. Een

logistische regressie analyse voor zwangerschap liet eveneens zien dat ovariële reactie op hyperstimulatie een hogere voorspellende waarde heeft dan leeftijd. Een strikte leeftijdsgrens voor behandeling met IVF dient dan ook niet gehanteerd te worden. In plaats hiervan kan de ovariële reactie op gecontroleerde hyperstimulatie gebruikt worden om patiënten met goede vooruitzichten op een succesvolle behandeling te selecteren.

Hoofdstuk 6 behandelt de behandelingsprognose na slechte bevruchting van eicellen in de eerste IVF-cyclus. Falende bevruchting en slechte bevruchting werden gedefinieerd. De relatie tussen bevruchtingsgraad en het aantal bewegende zaadcellen/ ml semen werd geanalyseerd. Drie patientengroepen met verschillende semenkwaliteit in de eerste IVF-cyclus werden gedefinieerd. In alle groepen bleek slechte bevruchting een hoge herhalingskans te hebben in een tweede cyclus. In de bestudeerde populatie dient het gebruik van de intra-cytoplasmatische spermatozoa injectie overwogen te worden na slechte bevruchting in de eerste IVF-cyclus. Dit geldt vooral wanneer sprake is van een kleine eicelopbrengst.

Hoofdstuk 7 is een overzicht van de literatuur over het vóórkomen van klinische complicaties gerelateerd aan de IVF-behandeling. In dit hoofdstuk worden tevens de klinische complicaties die zich voordeden bij 2495 opeenvolgende cycli in het transport IVF programma beschreven. Hierbij was sprake van vier verschillende complicaties leidend tot ziekenhuisopname: het ovariecl hyperstimulatiesyndroom, steeldraai van een adnex, salpingitis en extra-uteriene graviditeit. Een totaal aantal van 51 patienten werd met complicaties opgenomen. Het is aangewezen patienten voor te lichten over het vóórkomen van ernstige complicaties van IVF voor aanvang van de behandeling.

Hoofdstuk 8 beschrijft de uitkomst van alle drie- en vierlingzwangerschappen ontstaan in 2495 opeenvolgende IVF-behandelingen. Het vóórkomen van spontane reductie tot een twee- of éénlingzwangerschap, en acceptatie van selectieve embryo reductie werden bestudeerd. Van de 624 verkregen zwangerschappen bleken er 23 een drielingzwangerschap te zijn, éénmaal was sprake van een vierling. Selectieve embryoreductie werd door twee patienten met een drielingzwangerschap en door de patient met de vierling verkozen als behandelingsmogelijkheid, en werd dus door slechts 12.5% van de patienten geaccepteerd. Spontane reductie naar een tweelingzwangerschap kwam voor bij 3 van de drielingen. De 18 drielingen die een zwangerschapsduur van 20 of meer weken bereikten werden vergeleken met 54 tweelingen, willekeurig gekozen uit dezelfde populatie, vergelijkbaar voor wat betreft leeftijd en pariteit. De resultaten van IVF-behandeling na terugplaatsing van 2, 3 of 4 embryo's, en de kans op een

drielingzwangerschap na terugplaatsen van 3 embryo's werden geanalyseerd. Een hoge perinatale sterfte werd gevonden in de groep drielingen (11/54). Van de 18 paren met een drielingzwangerschap werden er 6 geconfronteerd met het verlies van tenminste één kind. In vergelijking met tweelingen werden drielingen vroeger geboren, hadden zij een lager geboortegewicht, en werden de patiënten en de kinderen vaker en langduriger opgenomen. Een zwangerschap na terugplaatsen van 3 embryo's bleek in 7.5% van de gevallen een drielingzwangerschap te zijn. Terugplaatsen van slechts 2 embryo's bleek acceptabele behandelingsresultaten te geven, en is het in het IVF-programma in Rotterdam gekozen beleid om drielingzwangerschappen te voorkomen.

In **hoofdstuk 9** worden de verschillende methodes om de succespercentages van een IVF-programma weer te geven besproken. De cumulatieve kans op zwangerschap na drie pogingen in het transport IVF-programma werd berekend. Onderzocht werd of deze cumulatieve kans beinvloed werd doordat relatief veel patienten met een slechte behandelingprognose het programma verlieten. Cycli van patienten die na een mislukte poging stopten met IVF werden vergeleken met cycli van patienten die doorgingen met de behandeling. Vergeleken werden de bevruchtingsgraad en de frequentie van vóórkomen van voorspellers van slechte uitkomst van de behandeling: eicelopbrengst ≤ 2 en terugplaatsing van < 2 embryo's. De cumulatieve zwangerschapskans na drie cycli was 55.1%. Patiënten die stopten met de behandeling en zij die doorgingen verschilden niet statistisch significant voor genoemde parameters. De cumulatieve zwangerschapskans van 55.1% na drie cycli geeft derhalve een betrouwbare indicatie van de kans op zwangerschap voor een willekeurig gekozen patiënt uit de bestudeerde populatie. De kans op zwangerschap bleek af te nemen in volgende cycli.

Conclusies

- Decentralisatie van een IVF programma met transport- en satellietklinieken heeft geen nadelig effect op de uitkomst van de behandeling en biedt de patiënten verschillende voordelen.

- In de meeste gevallen kan het monitoren van de gecontroleerde ovariële hyperstimulatie voor IVF beperkt worden tot slechts één echoscopie.

- Een leeftijdslimiet van 40 jaar voor deelname aan IVF-behandeling is niet gerechtvaardigd.

- Slechte bevruchting heeft een hoge kans op herhaling in een tweede IVF cyclus onafhankelijk van het aantal bewegende zaadcellen per ml semen in de eerste cyclus.

- In ongeveer 2% van de gevallen geeft IVF-behandeling klinische complicaties leidend tot ziekenhuisopname.

- Drielingzwangerschappen na IVF dienen voorkomen te worden door het terugplaatsen van slechts twee embryo's.

- De geldigheid van de cumulatieve zwangerschapskans dient getest te worden door een analyse van de resultaten van de patiënten die stopten met de behandeling.

Dankwoord

Op de eerste plaats wil ik mijn promotor Gerard Zeilmaker bedanken. Zonder zijn stuwende enthousiasme zou dit proefschrift niet geschreven zijn. Zijn voortdurende beschikbaarheid voor overleg en altijd opbouwende kritiek hebben me steeds gestimuleerd om door te gaan.

Arie Verhoeff, mijn co-promotor, die mij ooit aannam als arts-assistent na een sollicitatiegesprek in onvervalst Sesotho dat minder dan tien woorden vergde, stelde mij in de gelegenheid alle in het Zuiderziekenhuis beschikbare gegevens voor het onderzoek te gebruiken en analyseren. Onze gesprekken in de auto tussen Rotterdam en de Alblasserwaard over de meest eenvoudige en praktische uitvoering van de IVF behandeling (boeren-IVF volgens Arie) hebben mij zeker in het begin de weg gewezen. Arie, voor alles wat ik van je geleerd heb, en vooral ook voor je aanstekelijke enthousiasme, veel dank.

Voor hun bereidheid om zitting te nemen in de promotiecommissie wil ik graag Prof. Dr. J.W. Wladimiroff, Prof. Dr. P. Devroey, Prof Dr. M. Dhont en Prof. Dr. J.M.W.M. Merkus bedanken.

Mijn maat en paranimf Arne van Heusden heeft vanaf het begin een belangrijke rol gespeeld. Hij was het brein achter de database waarin alle gegevens werden opgeslagen. Zijn steun was onmisbaar bij de statistische bewerkingen. Heus, bedankt voor je vele adviezen en hulp. Harold Mous, veel dank voor het mede ontwikkelen van de database en de hulp bij het verzamelen van de gegevens.

Verder wil ik bedanken: Gerda Huisman van het IVF laboratorium Dijkzigt voor de samenwerking bij de totstandkoming van de eerste publikatie (hoofdstuk 1), Henk Bremer voor zijn rol als paranimf, Petra ten Hoope-Bender voor de taalkundige correctie van het manuscript, Jan Bos voor de adviezen voor de opmaak, en Leen Doornhein voor het maken van de omslag en de speciale aandacht voor het drukwerk.

Alle medewerkers van het IVF laboratorium van het Dijkzigt Ziekenhuis bedank ik voor hun aandeel in de behandeling van de patienten beschreven in dit proefschrift. Tenslotte is de kwaliteit van het IVF laboratorium naar mijn stellige overtuiging de belangrijkste succesbepalende factor bij de IVF behandeling.

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