CLINICAL ASSISTED REPRODUCTION

Incidence and Perinatal Outcome of Multiple Pregnancies After Intracytoplasmic Sperm Injection Compared to Standard In Vitro Fertilization

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Purpose: Our purpose was to assess the incidence of multiple pregnancies and their obstetric outcome after intracytoplasmic sperm injection.

Methods: The study group comprised women who delivered twins or triplets after intracytoplasmic sperm injection and standard in vitro fertilization. The incidence and main perinatal outcome of 140 multiple pregnancies resulting from intracytoplasmic sperm injection or standard in vitro fertilization treatment were analyzed.

Results: A total of 60 multiple pregnancies was obtained after intracytoplasmic sperm injection (3.4 ± 1.1 embryos/cycle) and 80 after standard in vitro fertilization (3.3 ± 2.0 embryos/cycle). The incidence of multiple pregnancy, i.e., 22.6 compared to 20.7%, respectively, was calculated. The obstetric outcome of 47 multiple pregnancies after intracytoplasmic sperm injection was 39 twin deliveries at between 27 and 37 weeks of gestation (mean, 36 ± 3.3) and 8 successful triplet deliveries between 26 and 36 weeks of gestation (mean 32.6 ± 2.4). The outcome after regular in vitro fertilization was similar. No major malformations were observed.

Conclusions: The results of this study showed that the incidence of multiple pregnancies after intracytoplasmic sperm injection was similar to that after standard, conventional in vitro fertilization. The perinatal outcome did not differ between both groups.

KEY WORDS: multiple pregnancies; intracytoplasmic sperm injection; in vitro fertilization.

INTRODUCTION

In vitro fertilization (IVF) treatment has a considerable influence on the incidence of multiple pregnancies. The increased medical risks entailed by high-order pregnancies are well known (1–3). The introduction of the intracytoplasmic sperm injection (ICSI) procedure for severe male infertility has increased the volume of IVF cycle treatment and the incidence and risk of multiple pregnancies (3–8).

A few investigators have reported the occurrence and obstetric outcome of multiple pregnancies. Moreover, there is evidence that multiple pregnancies in an IVF population are associated with a high perinatal mortality (9–11).

A 23%–30% incidence of twin pregnancies, and 4%–5% of triplets has been reported after IVF, having been calculated from the overall pregnancy rate (12,13).

However, the occurrence of multiple pregnancies after IVF and ICSI therapy has not been adequately investigated. This paucity of literature in specifically reporting the percentage and obstetric outcome in multiple pregnancies, especially in twin and triplet gestations after standard IVF, makes it difficult to provide patients with accurate counseling.

The aim of this study was to investigate whether the incidence and obstetric and neonatal outcomes of multiple pregnancies following IVF and ICSI differ when compared to the outcome after standard IVF.

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MATERIALS AND METHODS

During the period December 1994–October 1996, 1152 cycles of oocyte retrievals for IVF-ICSI treatment were analyzed at the IVF Unit, The Chaim Sheba Medical Center. The group of patients that enrolled in the standard IVF program during the same period served as the controls. The files of all patients and their cycle outcomes were summarized and analyzed. The protocol of ovulation induction comprised 3.2 mg of gonadotropin-releasing hormone (GnRH)- α (D-TRP₆; decapeptyl depot, controlled release; Ferring, Malmö, Sweden) given on day 1 of the cycle, followed 2 weeks later by 225 IU of human menopausal gonadotropin (hMG; Teva Pharmaceuticals, Kfar Saba, Israel) per day. After serum estradiol (E₂) concentrations reached >1835.5 pmol/L and at least three follicles >18 mm in diameter were observed on vaginal ultrasound, human chorionic gonadotropin (hCG; 10,000 IU; Chorigon, Teva Pharmaceuticals, Petah Tikva, Israel) was injected. Oocytes were retrieved by ultrasonographically guided vaginal puncture and were cultured and maintained in human tubal fluid medium supplemented with 10% scientific serum (Irvine Scientific, Irvine, CA) (14). Semen was considered abnormal if the sperm density was $<10 \times 10^6$ spermatozoa/ml, the sperm motility <40%, and the normal sperm morphology <14%, using strict criteria for morphology assessment. During the treatment cycle, some husbands (with a sperm count of $<0.5 \times 10^6$ /ml) were asked for two semen samples, the first on the morning of ovum pickup and the second on the same day after the procedure. The sperm selection procedure consisted of treatment on a Percoll or mini-Percoll gradient.

Oocytes were retrieved by vaginal ultrasound-guided oocyte pickup. About 1 h after retrieval, the cumulus and corona radiata were removed by incubation for <1 min in HEPES-buffered Earle's medium containing 160 or 80 IU/ml hyaluronidase (Type VIII; Sigma Chemical Co., St. Louis, MO). The surrounding cumulus and corona cells were further removed by aspiration in and out of a hand-drawn glass pipette of ~200- μ m diameter. The oocytes were then observed under the inverted microscope at $\times 400$ magnification to assess the integrity and maturation stage by observation of the presence or absence of the germinal vesicle or the first polar body. The oocytes were then further incubated for 3–4 hr and observed again to assess whether more oocytes had extruded the first polar body. Assisted fertilization was carried out on all morpholog-

ically normal oocytes that had extruded the first polar body.

Holding and injection pipettes were used (Cook, Spencer, IN). The outer and inner diameters of the holding and injection pipettes were 60 and 20 and 8 and 5 μ m, respectively. The injection pipette had a bevel angle of 50° and a sharp spike to assist penetration through the oolemma. A single spermatozoon, which was first immobilized, was aspirated tail-first into the tip of the microinjection pipette. During the ICSI procedure, the oocyte was held by the holding pipette with the polar body at the 12-o'clock or 6-o'clock position; the micropipette was pushed through the zona pellucida and into the ooplasm at the 3-o'clock position, and the single spermatozoon was injected into the ooplasm with 1–2 pl of medium. The injection pipette was withdrawn gently and the oocyte was released from the holding pipette (15,16).

After the microinjection procedure, the oocytes were incubated in 25- μ l microdrops of human tubal fluid medium. The state of oocyte fertilization was assessed 16–18 hr after the injection by looking for the presence of pronuclei. Fertilization was considered normal when two pronuclei were present. If embryos of sufficient morphologic quality were available, up to three embryos were placed into the uterine cavity ~48 hr after the microinjection procedure. Any extra embryos of good morphological quality were cryopreserved for possible later use.

Pregnancy was confirmed by detecting rising serum human choric gonadotropin concentrations on no fewer than two occasions at least 10 days after embryo replacement. Statistical analysis was performed using Student's *t* test.

After a patient was known to be pregnant, details of the course of the pregnancy and delivery, and information concerning the gestational sacs was recorded. The duration of the pregnancy was calculated from the day of embryo replacement considered as being the second week of gestation, up to the day of delivery or pregnancy termination. Abortion was defined as clinical pregnancy loss which occurred before week 20 of gestation. Perinatal mortality was defined as the sum of stillbirths plus infant mortality during the first 7 days after birth.

The abortions and deliveries were analyzed. The diagnosis of multiple pregnancies was made by transvaginal ultrasound examination at 7 weeks' gestation, and the presence of embryos with heart activity confirmed the diagnosis. Twin and triplet pregnancies were compared for complications during pregnancy, neonatal

tal mortality, and the number of multiple pregnancies in the ICSI-IVF population and standard IVF patients.

For statistical analysis we used Student's *t* test for the differences between two means, the χ^2 test to compare the frequencies of observations, and analysis of variance to compare the study groups: $P < 0.05$ was taken as the level of significance.

RESULTS

During the aforementioned period 652 clinical pregnancies were achieved by standard and ICSI-IVF: 387 after standard IVF therapy and 265 after ICSI-IVF treatment. A total of 140 (21.5%) clinical multiple pregnancies were obtained from the total number of pregnancies. Of 652 pregnancies, 80 were multiple after a standard IVF program was used, and 60 multiple pregnancies were achieved after the ICSI-IVF procedure. The abortion rate of multiple pregnancies was 33 of 140 (24%); 48 pairs of twins and 12 sets of triplets were born live after the standard IVF program. This included 7 sets of twins (reduced from quintuplets) and 1 twin (reduced from a triplet), compared to 39 pairs of twins and 8 sets of triplets born after the ICSI-IVF procedure, which included 6 sets of ICSI twins (reduced from quadruplets). Table I summarizes the incidence of multiple pregnancies after the ICSI-IVF procedure compared to standard IVF.

Table II shows the obstetric complications of multiple pregnancies. A trend toward increased pregnancy-induced hypertension in cases with triplets after standard IVF was observed.

Table III depicts the outcome of these pregnancies. A trend toward increased discordancy (≥ 500 g) between multiple pregnancies after ICSI-IVF was noted.

DISCUSSION

In vitro fertilization and ICSI have become widely accepted and a proven modality for the treatment of homogeneous groups, in which the majority of female patients are potentially fertile. The data on pregnancy outcome, especially the group of multiple pregnancy outcome after ICSI, are accumulated. Despite the fact that IVF patients are a heterogeneous, infertile group, a comparison with IVF-ICSI groups is important to achieve data on the outcome of ICSI-IVF pregnancies. Comparison between the ICSI group and the pregnancies obtained naturally is more difficult. The existing data on the outcome of pregnancies, especially high-order pregnancies, after a natural cycle compared to IVF cycles are scant (1,2). We are aware that ovarian stimulation, underlying infertility factors and the infertile status itself may carry a higher risk for pregnancy complications.

Our study demonstrated that the incidence of multiple pregnancies resulting from ICSI-IVF therapy and standard IVF is similar. The literature has shown that about 21%–23% of the pregnancies achieved after IVF-ET are multiple (3,5). Early pregnancy loss before 20 weeks of gestation was similar also in the ICSI group compared to the standard IVF group. The multiple pregnancy rate per embryos transferred was 5.2% for ICSI-IVF, compared to 5.4% for standard IVF. This indicates that implantation rates are similar, showing that oocytes originating from women with normal ovulatory cycles provided embryos of similar quality in both groups. An evaluation of the pregnancy outcome resulting from ICSI for severe male-factor infertility showed that there were no statistical differences, compared to patients who became pregnant and were delivered after a standard IVF program. No ectopic pregnancies were observed among the ICSI-IVF patients. A very low incidence of extrauterine

Table I. The Incidence of Multiple Pregnancies After Intracytoplasmic In Vitro Fertilization (IVF) Compared to Standard IVF

	Intracytoplasmic sperm injection	Standard in vitro fertilization	<i>P</i> value
Cycles (No.)	1152	1488	NS
Embryo transfers/cycle ^a	3.4 $\pm$ 1.1	3.3 $\pm$ 2.0	NS
Clinical pregnancies (%)	265 (23)	387 (26)	NS
Multiple gestations (%)	60 (22.6)	80 (20.7)	NS
Twins	39	48	NS
Triplets	8	12	NS
Abortions (multiple) (%)	13 (22)	20 (25)	NS

^a $\pm$ SD.

Table II. Obstetric Complications: Intracytoplasmic Sperm Injection (ICSI) Versus Non-ICSI in Multiple Pregnancies

	ICSI		Non-ICSI		P value
	Twins	Triplets	Twins	Triplets	
Maternal age ^a	31.8 ± 3.2	33.6 ± 2.4	35.1 ± 3.8	32.8 ± 3.0	NS
Primigravidae (%)	27 (69)	5 (63)	26 (54)	9 (75)	NS
Antenatal hospitalization (%)	46	63	50	58	NS
Pregnancy-induced hypertension (%)	26	25	29	41	0.39
Premature rupture of membranes (%)	44	38	46	42	NS
Cesarean section (%)	59	100	54	100	NS
Weeks of delivery					
26–34	11	5	13	7	NS
35–38	28	3	35	5	NS

^a ± SD.

pregnancy, less than 1%, was observed by others (1), logically attributable to the tubal factor.

Most of the recent literature concerning multiple pregnancy outcome has reported data from regular, standard IVF programs (1–13). Few studies have solely reported on the outcome of ICSI–IVF pregnancies, especially multiple pregnancies (1). Since the widespread application of the ICSI technique has raised concerns about the efficacy and safety of this technique, some authors reported an increased incidence of multiple pregnancy and a slight increase in the incidence of chromosomal abnormalities. This supports the need to advise these couples properly and to continue follow-up of the newborns to facilitate accurate evaluation of the risks after ICSI. On evaluation of the pregnancy outcome in our study, we noted that the abortion rate was not elevated after ICSI–IVF was performed, and this is important when counseling patients, particularly anxious, infertile couples. Our work elucidates this field. Obstetric complications in general increase with the growing number of fetuses. When analyzing the outcome, we observed that prema-

ture labor and the cesarean section rate did not differ between ICSI–IVF and standard IVF (Table II). However, a trend toward twin discordancy was observed in the ICSI group: 33.3% in the twins and 12.5% in the triplets of this group, compared to 20.8% and 8.3% in the non-ICSI group, respectively. Another study demonstrated an increased rate of discordant birth weights among patients after IVF treatment compared to spontaneous multiple gestations (5). This is an atypical phenomenon for dizygotic twins because of their separate chorionic membranes. It is possible that genetics play a major role in this phenomenon. Furthermore, the duration of hospitalization was no longer in the group that achieved pregnancy after ICSI–IVF. It was shown that routine hospital admission for multiple pregnancies was not necessarily beneficial in prolonging the gestation (17,18). The frequency of premature rupture of the membranes (PROM) was 44% in ICSI twin and 38% in ICSI triplet pregnancies and did not differ compared to the standard IVF group (Table II). Because most of the neonates of pregnancies with PROM are delivered on the same day, it is important,

Table III. Outcome—Multiple Pregnancy Comparisons Between Intracytoplasmic Sperm Injection (ICSI) and Non-ICSI Procedures

	ICSI		Non-ICSI		P value
	Twins	Triplets	Twins	Triplets	
Gestational age ^a	35.7 ± 3.3	33.6 ± 5.4	35.0 ± 3.5	32.8 ± 5.0	NS
Birth weight (g) ^a	2219.1 ± 582.9	1549.8 ± 596.8	2176.5 ± 636.6	1502.6 ± 547	NS
<1599 (newborns)	11	11	13	20	NS
>2500 (newborns)	32	1	45	1	NS
Hospitalizations	18 (39)	5 (8)	24 (48)	7 (12)	NS
Neonatal mortality	2	2	5	3	NS
Malformations	1	2	1	1	NS
Discrepancy (%)	13 (33.3)	1 (12.5)	10 (20.8)	1 (8.3)	Twins, 0.14 Triplets, 0.65

^a ± SD.

when counseling patients in whom this complication occurs, to emphasize that the occurrence of PROM is not increased in the ICSI multiple-gestation group. The rate of PIH showed a trend toward a high percentage in the non-ICSI triplet group but not when we analyzed the twin gestations. It has also been observed that the occurrence of PIH in twins was substantially lower than that reported in other populations (19–21).

The mortality rate and neonatal malformations were not increased in the ICSI multiple-pregnancy group (Table III). Surprisingly, we observed a very low neonatal mortality in the ICSI group, but on statistical evaluation, no difference existed between multiple pregnancy/neonatal mortality in both groups. However, authors from our institute (22) calculated the perinatal and neonatal mortality rates of 78 triplet pregnancies. There were 93 of 1000 and 51 of 1000, respectively, but only 9% of patients achieved pregnancy after IVF. An important observation of our study is that the mortality rate of the neonates is not increased. These data support the observations of others (1,5) when comparing ICSI to standard IVF.

While evaluating neonatal malformation, we also found no statistical differences between the outcome of ICSI–IVF and that of regular IVF. These data support the notion that the overall incidence of major congenital anomaly detected in the first week of life for the newborns conceived after IVF–ICSI are not higher than the data reported after standard IVF outcome. Only one newborn of the non-ICSI triplets had mild VSD, compared to one ICSI twin with the same malformation. Two newborns of the ICSI triplets had other malformations, one skeletal and one diaphragmatic hernia.

Since the number of multiple pregnancies, and mainly triplets, was relatively high in our series, we adopted a novel approach of transferring up to three embryos, even in patients who fail a substantial number of IVF treatments.

In summary, comparing our ICSI–IVF patients with multiple gestations to patients treated by regular IVF, we found a similarity in the incidence of multiple pregnancy. The abortion rate was comparable. Moreover, so far, the outcome of these pregnancies after evaluating different parameters appear to be similar. These data support previous observations (1) that the ICSI–IVF multiple pregnancies have no increased risk of congenital anomalies or perinatal mortality. Pregnancy complications occurred at the same frequency in the ICSI–IVF group as in the standard IVF program.

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