Results of Intracytoplasmic Sperm Injection of Single Oocyte in 362 Unstimulated Cycles

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Purpose: The purpose was to test the adequacy of unstimulated cycles for intracytoplasmic sperm injection (ICSI) and to evaluate implantation and pregnancy rates in three different age groups.

Methods: ICSI was performed in 362 unstimulated cycles in women up to 45 years old. All cycles were monitored by serum estradiol, urinary LH, and ultrasound.

Results: The delivery rate per started cycle was higher in patients younger than 36 (9.4%) than in cycles with patient's age between 36 and 39 years (4.8%) or older than 40 (4.6%) but the difference was not statistically significant. In all groups, the fertilization rate was similar (70.4, 77.6, and 84.8%, respectively). The pregnancy rate per cycle and the pregnancy rate per puncture was similar in the group of patients in age between 36 and 39 years (8.3 and 9.7%) and those older than 40 (7.7 and 9.3%).

Conclusions: Unstimulated cycles monitored by serum estradiol, urinary LH, and ultrasound can produce an acceptable pregnancy rate after ICSI procedure only in patients younger than 36.

KEY WORDS: Intracytoplasmic sperm injection; monitoring; unstimulated cycle.

INTRODUCTION

The first knowledge on procedures of in vitro fertilization (IVF) was collected in unstimulated cycles, resulting in the first successful pregnancy in an unstimulated cycle (1). Simplification of laboratory procedures and monitoring were the reasons for the return of spontaneous cycles in the beginning of the nineties (2,3). Apart from ultrasonographic following of follicle growth, the simplification of natural cycle monitoring also implies the introduction of rapid tests for determining serum estradiol (E2) and rapid tests for the presence of luteinizing hormone (LH) in urine.

In timing of oocyte pick up in unstimulated cycles for IVF, most authors apply the administration of human chorionic gonadotrophin (hCG) (4–7) or detection of natural LH surge (8–11).

Acceptable pregnancy rates in unstimulated cycles, comparable with natural fecundability of fertile couples, were reported in several groups (8,9). Relatively high pregnancy rates per embryo transfer (20%) similar to natural fecundability were reported less frequently (2) than lower values (6,7).

Unstimulated cycles proved ineffective when IVF was used in the treatment of male factor infertility (8,12,13). That is why only individual cases of unstimulated cycles in the treatment of severe cases of male infertility with ICSI can be found in the literature (14).

MATERIALS AND METHODS

The study of candidates for ICSI procedure in unstimulated cycles included patients with ovulatory

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cycles who were younger than 45. During this prospective nonrandomized study all consecutive patients were followed with the same protocol during the same period of study. Folliculometry was done with a 5-MHz vaginal probe (Aloka 1700). The first ultrasound examination was done on the second day of the menstrual period. After that follicular growth was monitored by serum estradiol only on Day 5 and Day 7 of the menstrual cycle. At the moment when the estradiol level reached 0.49 nmol/L, follicular diameter was measured. When the mean follicular diameter reached 15 mm or more together with an estradiol level of 0.49 nmol/L, the criteria for hCG application were fulfilled.

The presence of urinary LH in the morning urine was checked by the Rapi Test LH (Morwell, Switzerland). A positive test on the day before hCG application was an indication for cycle cancellation.

Follicle aspiration was done 35 h after hCG application. Only the dominant follicle was aspirated. In case no oocyte was found in the aspirate, the follicle was rinsed and the aspirate reexamined.

Following retrieval, the oocytes were exposed to 80 IU/mL hyaluronidase for 6–10 s (MediCult, Denmark). Surrounding cumulus cells were stripped from oocytes by aspiration through a pipette. Oocytes that were in metaphase II (MII) were injected immediately after the procedure. Sperm injection was performed after sperm immobilization using polyvinylpyrrolidone (PVP) (MediCult, Denmark). ICSI was performed on an inverted microscope (Olympus, Japan) and Narishige micromanipulators with micropipettes (Hunter Scientific, England). Injected oocytes were rinsed and placed in a droplet of culture universal IVF medium (MediCult, Denmark) covered by liquid paraffin (MediCult, Denmark). Fertilization was assessed 15–18 h after insemination. On Day 2 of culture, embryo cell number and morphology were scored. Adequate embryos were transferred using soft embryo transfer catheter (Cook, Australia and Labotect, Germany).

Serum estradiol levels were measured by enzymeimmunoassay AxSYM Estradiol assay (Abbott). The conversion factor for estradiol from SI unit is 1 nmol/L = 272.4 pg/mL. The luteal phase was supported by 1500 IU of hCG (Pregnyl, Organon, Holland) immediately after oocyte pick up.

Implantation was confirmed by determination of serum beta hCG 16 days after oocyte pick up. Pregnancy was confirmed as clinical only in case of ultrasonic evidence of a gestation sac. All pregnant women

continue with luteal supplementation with 30 mg of dydrogesteron daily (Dabroston, Duphar, Holland) until the eighth week of pregnancy.

The data were processed with the statistical program by Statistica (StatSoft, USA). Analysis of discrete variables was done by chi-square analysis and Student's *t* test was used for parametric analysis.

Statistical significance was set at $p < 0.05$. The study was approved by the Ethics Committee at the Ministry of Health of the Republic of Slovenia.

RESULTS

On the day of hCG administration the mean follicle diameter was 15.4 ± 1.2 mm. The mean value of E2 on the day of hCG application was 0.72 ± 0.21 nmol/L. At follicle aspiration no oocyte was obtained in 36/213 (16.9%) of aspirates in the group of patients younger than 36, 6/62 (9.6%) in cycles with patient's age between 36 and 39 years, and 7/43 (16.2%) in the group of 40 and older. In the group of patients younger than 36 oocyte pick up was cancelled in 10.5% of cycles after hCG was administered, because ovulation occurred before aspiration. In both groups of patients older than 36 or 40 years this finding was observed in 13.8% and 17.3% of patients undergoing ICSI procedure. The difference was not statistically significant. The rate of normally developed embryos on Day 2 two days after puncture was similar in all ICSI groups (62.2, 62.1, and 71%). The implantation rate per embryo available for transfer was higher in the youngest ICSI group 27/114 (23.6%) when in patients older than 36 (10/63, 15.8%) (see Table I).

The pregnancy rate per cycle did not differ significantly between cycles with patient's age between 36 and 40 and older than 40. The oldest patient conceived after ICSI in unstimulated cycle was 44 years old. The oldest patient conceived and delivered after ICSI in unstimulated cycle was 43 years old at the moment of implantation.

In all groups 95.8% (177/185) of all fertilized oocytes were developed in embryos available for transfer on Day 2. The implantation rate of embryos was 20.9% (37/177). There were more miscarriages in the first trimester of pregnancy in older than 36. The take home baby rate per punctured cycle in both groups older than 36 and 40 was 4.8% and 4.6%. These differences were not statistically significant.

Table I. Results of the Outcome of Intracytoplasmic Sperm Injection of Single Oocyte in 362 Cycles

	Age (year)		
	<36	36–39	40–45
Started cycle	238	72	52
Premature ovulation after hCG	25 (10.5%)	10 (13.8%)	9 (17.3%)*
Oocyte pick up cycle	213	62	43
Cycles with oocytes (recovery rate)	177	56	36
Oocytes	182	58	38
Successfully denuded oocytes	178	54	37
MII oocytes	169	49	33
Normally fertilized oocytes			
2PN	119 (70.4%)	38 (77.6%)	28 (84.8%)*
Abnormally fertilized oocytes			
1PN	8	1	1
3PN	0	1	0
Embryos on Day 2	114 (62.6%)	36 (62.1%)	27 (71.0%)*
Clinical pregnancy	27	6	4
Ectopic pregnancy	1	0	0
Miscarriage in first trimester	6 (22.2%)	3 (50.0%)	2 (50.0%)
Live birth (deliveries)	20	3	2
Pregnancy rate per embryo available for transfer	23.7%*	16.7%	14.8%
Pregnancy rate per puncture	12.7%	9.7%	9.3%
Pregnancy rate per started cycle	11.3%	8.3%	7.7%
Delivery rate per puncture	9.4%*	4.8%	4.6%

*Not significant.

DISCUSSION

Natural cycles for IVF-ET have gained increasing attention among patients in recent years. The possibility of the assisted reproduction procedure being carried out in a natural cycle, with an oocyte whose development is not the result of medication, seems more natural and acceptable for the patients. Women over 40 and couples with male factor infertility problems would not be advised to participate in unstimulated IVF cycles (2,8,10,13). Data reviewed in the literature advocate this with a very low pregnancy rate in such cases.

The results of numerous newer studies on unstimulated cycles in the IVF procedure indicate considerable progress in recent years, associated with better monitoring of follicle development. A pregnancy rate of up to 19.8% per embryo transfer (8) demonstrated that acceptable results can be obtained with-

out ovulation induction. Important limitation of this procedure is still the high cancellation rate (between 15 and 27.7%) (8). Monitoring of follicle development in nonstimulating cycles for IVF with ultrasound only and the detection of LH surge leads to 49.8% of canceled cycles (15). Using similar protocol of monitoring, in 63 women treated for tubal infertility we had 20.6% canceled cycles after hCG application, 18% pregnancies per embryo transfer, and 12% per puncture (13).

To give as much consideration as possible to the dynamics of follicular growth and physiologic differences in E2 secretion, an attempt was made to develop more individual criteria for evaluating the most suitable time for hCG application in unstimulated cycle. Paulson *et al.* (16) suggested that in a larger follicular diameter, a lower E2 level is required. In evaluating follicular maturity in unstimulated cycles prior to hCG application, some authors give adequate serum E2 level precedence over ultrasonographic measurement of follicular diameter. According to Foulot *et al.* (2), a follicle is mature when the follicle diameter exceeds 18 mm and serum estradiol exceeds 0.66 nmol/L. Despite the fact that the value of combining the criteria of maximum follicular diameter and serum E2 level is evident, it has not yet been agreed which E2 level is closest to the optimum. These values vary between 1.1 nmol/L (4), 0.73–1.1 nmol/L (17), 0.88 nmol/L (18), 0.50 nmol/L (19), and 0.40 nmol/L (20).

On the basis of results of our previous study (13), we lowered the criteria for hCG application to those when the follicle reaches at least 15 mm in mean diameter and serum E2 is 0.49 nmol/L (19). In spite of such low values of serum E2 and small mean follicle diameter, aspirated oocytes were suitable for ICSI with similar fertilization rate and implantation rate of cleavage embryos. Relatively low serum E2 levels prior to hCG application can decrease the cancellation rate and improve the recovery rate without an unfavorable effect on fertilization rate and pregnancy rate.

Accurate determination of follicle maturity on the basis of serum E2 level is very difficult. Observation of the onset of the preovulatory LH surge in unstimulated cycle showed that the relationship between follicle size and serum E2 is not sufficiently strong to predict the LH surge. The LH surge is unlikely to occur either before the follicle diameter has reached 15 mm and/or serum E2 level has reached 0.60 nmol/L. These results suggest that hCG could be given earlier

in the cycle. In our previous study (19) a comparison of implantation rates in unstimulated IVF/ICSI cycles with the dependence on different E2 levels prior to hCG application reveals a statistically significantly higher implantation rate in cases when serum E2 level was lower than 0.9 nmol/L (71.4%) than in cases when E2 level was higher than the mentioned values (13.9%). In cases when the E2 level prior to hCG application was higher than 1.1 nmol/L, there was no embryo implantation after embryo transfer. Nevertheless, the fertilization rate was independent of E2 levels.

With this approach the pregnancy rate was not compromised but the cancellation rate was significantly decreased. Using this procedure in our study, we decreased the percentage of canceled ICSI cycles to only 10.5%, in patients younger than 36, the fertilization rate in ICSI, however, remained high, amounting to 70.4%. The pregnancy rate per embryo transfer in ICSI cycles was 23.7%.

In the literature we found no study evaluating the success of the ICSI procedure in unstimulated cycles. This study confirms the expectations that ICSI in unstimulated cycles can generate similar results as those obtained in IVF unstimulated cycles. The main hypothetical disadvantage of ICSI of single oocyte is possibility for its mechanical damage during this technically complicated procedure for fertilization. The advantage of the method is the expectation that better results of fertilization are related to possibility of identifying mature oocytes in ICSI cycles. Unfortunately in the literature there are no publications on this topic. All results are related to IVF procedure in unstimulated cycles. Our results show that the success of the ICSI procedure in patients younger than 36 is equal to that of the IVF procedure in unstimulated cycles. Performing a prospective study allocating unstimulated patients to each procedure in a randomized fashion would provide a definitive proof of superiority of ICSI versus IVF. ICSI in unstimulated cycles has all elements of simplification, the result being a safer and more comfortable procedure for the patients and faster, less time consuming, and less risky treatment for the ICSI center.

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