Spermatogenic Patterns and Early Embryo Development After Intracytoplasmic Sperm Injection in Severe Oligoasthenozoospermia

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Purpose: Evaluate the influence of different baseline spermatogenic patterns [meiotic pattern (normal or abnormal), sperm concentration ($>1 \times 10^6/\text{mL}$ or $\leq 1 \times 10^6/\text{mL}$), and the combined meiosis-sperm concentration pattern] on early embryo development in severe oligoasthenozoospermia.

Methods: Embryo outcomes (fertilization rate, cleavage rate, and 4-cell stage embryo division rate on day 2) after IVF-ICSI in 75 oligoasthenozoospermia and 79 normozoospermic males.

Results: The embryo division rate was significantly lower in oligoasthenozoospermia compared to normozoospermia (50.43% vs. 58.72%, $p < 0.01$) and in the oligoasthenozoospermia group for meiotic anomalies (43.40%), sperm concentration $\leq 1 \times 10^6/\text{mL}$ (44.35%), and the combined pattern $\leq 1 \times 10^6/\text{mL}$ with meiotic anomalies (37.17%). Logistic regression analysis showed a synergic effect (OR = 2.00; 95% CI = 1.28–3.12) when the two spermatogenic patterns predictive of slow embryo development [meiotic anomalies (OR = 1.49; 95% CI = 1.03–2.15) and sperm concentration $\leq 1 \times 10^6/\text{mL}$ (OR = 1.53; 95% CI = 1.09–2.13)] were present.

Conclusions: The data suggest that the early embryonic developmental capacity is inversely related to the severity of spermatogenic impairment (meiotic anomalies and/or sperm concentration $\leq 1 \times 10^6/\text{mL}$).

KEY WORDS: Early embryo development; intracytoplasmic sperm injection; meiotic chromosome anomalies; oligoasthenozoospermia; sperm concentration.

INTRODUCTION

In a previously studied series of men with idiopathic severe nonobstructive oligoasthenozoospermia who met the criteria for intracytoplasmic sperm

injection (ICSI), evaluated by means of testis biopsy and then with a quantitative/qualitative analysis of their spermatogenic process, we examined both the incidence of meiotic anomalies (1) and their relationship with different quantitative spermatogenic parameters (2). Meiotic anomalies included severe meiotic arrest (presence of pachytenes and occasional spermatozoa, but no metaphase I figures found) and synaptic anomalies (chromosome pairing anomalies). Severe meiotic arrest due to synaptic anomalies or to unknown causes results in a incomplete spermatogenic arrest. Synaptic anomalies, limited to the germ cell line in males with normal karyotype, are

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usually associated with a severe meiotic arrest. A high incidence of meiotic anomalies (38.1%), especially synaptic anomalies (17.6%), was observed (1), and a sperm concentration $\leq 1 \times 10^6/\text{mL}$ or an increased baseline serum FSH level were found to be predictive risk factors for meiotic anomalies (2).

To date, few studies have analyzed the early embryo development after ICSI in oligoasthenozoospermia with respect to spermatogenic parameters (3,4) and in no case the study included the meiotic patterns.

The objective of the present controlled study was to evaluate the influence of different baseline spermatogenic patterns [meiotic pattern (normal or with meiotic anomalies), sperm concentration ($>1 \times 10^6/\text{mL}$ or $\leq 1 \times 10^6/\text{mL}$), and the combined meiosis-sperm concentration pattern] on early embryo development (fertilization and cleavage rates, 4-cell stage embryo division rate on day 2) after ICSI in patients with severe nonobstructive oligoasthenozoospermia.

MATERIALS AND METHODS

Patients

Seventy-five patients from the previously-studied sample who underwent between February 1998 and May 1999 their first ICSI cycle in the year following their initial consultation in our andrology department, and with clinically normal female (ovulatory, tubal, endometrial, and cervical) factor, were included in the present study. In all patients a baseline spermatogenic study including clinical history, semen analysis and culture, serum FSH level, and testis biopsy was performed, as described previously (2). Seminal samples were assessed for sperm concentration, sperm motility percentage, and motile sperm concentration by means of the Makler chamber and a previously described multiple exposure photography method (2), and each of them showed severe oligoasthenozoospermia (motile sperm concentration $\leq 1.5 \times 10^6/\text{mL}$) with low sperm concentration and low sperm motility. The cause of oligoasthenozoospermia was idiopathic or cytogenetic, according to clinical and histological data. The ICSI protocol was approved by the Ethical Committee of Institut Universitari Dexeus and written informed consent was obtained from all patients.

A control group of 79 normozoospermic (5) males who were undergoing IVF (by tubal female factor) at the same period of time was included in this study.

IVF and ICSI Procedures

Ovulation stimulation was carried out in all cycles using FSH (Neofertinorm®, Lab. Serono, Spain) following hypophyseal inhibition with a gonadotropin-releasing hormone analogue (leuprolide acetate; Procrin®, Lab. Abbott, Spain), according to a previously described long protocol (6). Pituitary suppression is started in the midluteal phase of the previous cycle with a leuprolide acetate s.c. daily dose of 1 mg. This dose is reduced to 0.5 mg/day once ovarian inhibition has been achieved, and is then continued until the administration of HCG. Ovarian response was monitored by vaginal ultrasound and serum estradiol levels, as previously described (6). Thirty-six hours after administration of 10,000 UI i.m. or s.c. of human chorionic gonadotropin (HCG; Profasi®, Lab. Serono, Spain), oocytes were retrieved by vaginal ultrasound-guided puncture.

The laboratory protocol for the preparation of semen and oocytes for IVF, including ICSI, and for sperm microinjection, used at the time period of the study in our IVF-ICSI program, has been described previously in detail (7).

Sixteen to 18 h after sperm microinjection, oocytes were observed to check for fertilization (2PN) and, 24 h later, embryos were evaluated according to criteria of division and number of cells (8). Two days after oocyte retrieval a maximum of three (exceptionally four) cleaved embryos were transferred. The remaining transferable embryos were cryopreserved.

Data Set and Statistical Analysis

In the oligoasthenozoospermia group, the independent spermatogenic variables were dichotomized as follows: meiotic pattern [normal (N) or meiotic anomalies (AN)] and sperm concentration ($>1 \times 10^6/\text{mL}$ or $\leq 1 \times 10^6/\text{mL}$).

In order to study trends, a new category was created out of the dichotomous variables meiotic pattern and sperm concentration: this was termed combined pattern ($>1 \times 10^6$ N, $\leq 1 \times 10^6$ N, $>1 \times 10^6$ AN, $\leq 1 \times 10^6$ AN).

The dependent embryonic variables were also defined as dichotomies: fertilization (present or absent), zygote division (present or absent), and embryo division rate [4 cells (normal) or <4 cells (slow)] on day 2.

Either the Student's *t*-test or the analysis of variance (ANOVA) test was used for analysis of the quantitative variables while the chi-square test was used

Table I. General Characteristics of the Patients

	Mean (SEM)		<i>p</i> ^a
	Normozoospermia (<i>n</i> = 79)	Oligoasthenozoospermia (<i>n</i> = 75)	
Man's age (years)	35.84 (0.48)	34.91 (0.55)	NS
Length of infertility (years)	5.07 (0.39)	4.48 (0.43)	NS
Sperm concentration ($\times 10^6/\text{mL}$)	79.95 (6.98)	2.91 (0.46)	<0.001
Woman's age (years)	34.04 (0.37)	32.93 (0.44)	NS
Oocytes inseminated	10.76 (0.66)	10.79 (0.65)	NS

^a Student's *t*-test.

for qualitative variables. The predictive value of the significant variables was evaluated by means of logistic regression analysis. The significance level was set at 0.05. The analyses were carried out using SPSS for Windows (SPSS, Inc, Chicago, USA).

RESULTS

Study Population

The general characteristics of the 75 couples studied and the control group couples are shown in Table I.

In the oligoasthenozoospermic patients group, sperm concentration ranged between $0.01 \times 10^6/\text{mL}$ and $10.6 \times 10^6/\text{mL}$. In 34 (45.33%) patients it was $\leq 1 \times 10^6/\text{mL}$. Motility ranged between 0 and 40.13% and motile sperm concentration ranged between 0 and $\leq 1.5 \times 10^6/\text{mL}$.

Frequency distribution for the meiotic pattern was 53 (70.67%) with normal and 22 (29.33%) with meiotic anomalies. The meiotic anomalies observed were 16 severe meiotic arrests and 6 synaptic anomalies (1 case of complete desynapsis, 2 cases of desynapsis affecting a variable number [2–5] of bivalents, and 3 cases with the presence of a variable number [2–6] of small univalents in their metaphase I figures).

The age of patients ranged from 26 to 59 years and the period of infertility between 1 and 22 years. The age of the women ranged between 21 and 40 years and the number of oocytes microinjected between 2 and 23.

In the control group, sperm concentration ranged between $22.5 \times 10^6/\text{mL}$ and $188.5 \times 10^6/\text{mL}$. The age of patients ranged from 25 to 48 years and the period of infertility between 2 and 18 years. The age of the women ranged between 27 and 40 years and the number of oocytes inseminated between 2 and 28.

No significant differences were found regarding the man's age, the infertility period, the woman's age, or the number of oocytes inseminated per cycle (normal ovulatory factor).

Overall Embryo Outcomes After IVF–ICSI

Table II shows overall embryo outcomes in both groups.

In the normozoospermic patients group, a total of 850 oocytes were inseminated, of which 625 became fertilized (73.53%); 587 zygotes divided (93.92%) and 367 embryos (58.72%) reached the 4-cell stage on day 2.

In the oligoasthenozoospermic patients group, a total of 809 oocytes were microinjected, of which 575 became fertilized (71.08%); 538 zygotes divided (93.57%) and 290 embryos (50.43%) reached the 4-cell stage on day 2.

No significant differences were found in fertilization and cleavage rates. When both groups were compared, a slower embryo division rate was found in men with severe oligoasthenozoospermia (50.43% vs. 58.72%, $p < 0.01$).

Table II. Overall Embryo Outcomes (%)

	Normozoospermia	Oligoasthenozoospermia	<i>p</i> ^a
Oocytes inseminated	850	809	
Oocytes fertilized	625 (73.53)	575 (71.08)	NS
Cleaved embryos	587 (93.92)	538 (93.57)	NS
Embryos 4 cells	367 (58.72)	290 (50.43)	<0.01

^a Chi-square test.

Table III. Embryo Outcomes (%) According to Spermatogenic Patterns

	Meiotic pattern			Sperm concentration		
	Normal	Abnormal	<i>p</i> ^a	>10 ⁶ /mL	≤10 ⁶ /mL	<i>p</i> ^a
Oocytes microinjected	579	230		452	357	
Oocytes fertilized	416 (71.85)	159 (69.13)	NS	336 (74.34)	239 (66.95)	<0.05
Cleaved embryos	387 (93.03)	151 (94.97)	NS	323 (96.13)	215 (89.96)	<0.01
Embryos ≥4 cells	221 (53.12)	69 (43.40)	<0.05	184 (54.76)	106 (44.35)	<0.05

^aChi-square test.

Embryo Outcomes According to Spermatogenic Patterns After ICSI

When compared with the normozoospermic group, no significant differences were found in fertilization, cleavage and embryo division rates for normal meiotic pattern, sperm concentration >1 × 10⁶/mL, and the combined pattern >1 × 10⁶/mL with normal meiosis.

In the oligoasthenozoospermic group, embryo outcomes after ICSI were analyzed according to the spermatogenic patterns described. No significant differences were found regarding the man's age, the infertility period, the woman's age, or the number of oocytes microinjected per cycle.

Table III shows embryo outcomes after ICSI according to the dichotomized spermatogenic patterns. A slower embryo division rate (43.40%, *p* < 0.05) was found with respect to the abnormal meiotic pattern although there were no differences in fertilization and cleavage rates. With respect to sperm concentration, both fertilization (*p* < 0.05) and cleavage (*p* < 0.01) rates were lower and the embryo division rate was also slower (44.35%, *p* < 0.05) for sperm concentration ≤1 × 10⁶/mL.

Table IV shows embryo outcomes for the combined meiosis-sperm concentration patterns. While there were no differences between the four patterns in terms of fertilization and cleavage rates, there was a significant trend toward slower embryo division as the sperm pattern became more severe (54.14% vs. 37.17%, *p* < 0.01).

The predictive relative risk of slow early embryo development for the significant spermatogenic patterns was 1.49 (95% CI = 1.03–2.15) for meiotic anomalies and 1.53 (95% CI = 1.09–2.13) for sperm concentration ≤1 × 10⁶/mL, and a synergic effect was observed (OR = 2.00; 95% CI = 1.28–3.12) when both risk patterns were combined. The multivariate model also confirms a synergism (*p* = 0.002) when both patterns are simultaneously present.

DISCUSSION

To date, few studies have analyzed embryo outcomes after ICSI in oligoasthenozoospermia with respect to isolated baseline spermatogenic parameters (4) and none of the studies included the meiotic patterns. Other studies have analyzed outcomes according to quantitative sperm parameters in the ICSI semen sample (3).

The present study analyzes embryo outcomes obtained after a first ICSI cycle according to different baseline spermatogenic patterns (meiotic pattern, sperm concentration, and the combined meiosis-sperm concentration pattern) in a previously studied (2) group of men with normal—but near the upper limit—baseline serum FSH level (mean 9.57 IU/L) in whom the quantitative histological diagnosis had confirmed a severe idiopathic oligozoospermia (average count of mature spermatids: 5.35). The previous baseline study found a high incidence of meiotic

Table IV. Embryo Outcomes (%) According to the Combined Meiosis-Sperm Concentration Pattern

	Combined pattern				<i>p</i> ^a
	>1 × 10 ⁶ N	≤1 × 10 ⁶ N	>1 × 10 ⁶ AN	≤1 × 10 ⁶ AN	
Oocytes microinjected	388	191	64	166	
Oocytes fertilized	290 (74.74)	126 (65.96)	46 (71.87)	113 (68.07)	NS
Cleaved embryos	278 (95.86)	109 (86.51)	45 (97.83)	106 (93.81)	NS
Embryos ≥4 cells	157 (54.14)	64 (50.79)	27 (58.69)	42 (37.17)	< 0.01

Note. N = normal meiosis; AN = meiotic anomalies.

^aChi-square for trend.

anomalies (38.1%) in this population (1), which increased (57.8% when the sperm concentration was $\leq 1 \times 10^6/\text{mL}$ and 68% when it was $\leq 0.5 \times 10^6/\text{mL}$) with the severity of spermatogenic impairment (2). In our opinion the baseline study not only provides etiopathogenic information, but avoids both physiological intraindividual variability in the semen analysis (5) and any possible bias in the ICSI semen sample due to the stress of the cycle (9).

Although the ideal control group was of normozoospermic males who underwent ICSI for female tubal factor at the same time as the patterns studied, it is known that it is difficult to have couples undergoing ICSI for female tubal factor. As the criteria of embryonic evaluation are the same in both IVF and ICSI cycles, the study includes, for comparison, a control or reference group of normozoospermic males who underwent IVF (by tubal female factor) at the same period of time.

All groups were comparable with respect to woman's age and number of oocytes microinjected per cycle (normal ovulatory factor).

In overall terms, when compared with the normozoospermic group, the rates of fertilization (71.08%) and zygote division (93.57%) were normal, while the embryo division rate on day 2 (50.43%, $p < 0.01$) was slower, as would be expected in men with severe oligoasthenozoospermia (10).

A lower fertilization rate was only found with sperm concentrations $\leq 1 \times 10^6/\text{mL}$ ($p < 0.05$). Other authors (3) have found a reduced fertilization rate ($p < 0.001$) for very low total sperm counts in the ICSI semen sample, with no differences in terms of the serum FSH level (4).

A significantly slower division rate on day 2 with predominance of slow embryos was found for meiotic anomalies (56.60%, $p < 0.05$), sperm concentration $\leq 1 \times 10^6/\text{mL}$ (55.65%, $p < 0.05$), and, especially, the combined pattern, i.e., $\leq 1 \times 10^6/\text{mL}$ with meiotic anomalies (62.83%, $p < 0.01$); there was a significant trend toward slower embryo division as the severity of spermatogenic impairment increased, with a synergic effect (OR = 2.00; 95% CI = 1.28–3.12) when the two spermatogenic patterns predictive of slow early embryo development [meiotic anomalies (OR = 1.49; 95% CI = 1.03–2.15) and sperm concentration $\leq 1 \times 10^6/\text{mL}$ (OR = 1.53; 95% CI = 1.09–2.13)] were simultaneously present.

Some authors have suggested that there is an important paternal factor in early embryo development in that severe oligoasthenozoospermia reduces the developmental capacity of the embryo (10,11), which

in turn leads to slow early embryo division and an increased likelihood of arrest (12), generally at the time of genome activation (10,13).

It is also known that sperm meiotic chromosome anomalies are usually associated with oligoasthenozoospermia and with the production of spermatozoa with a high rate of numerical chromosome anomalies (diploidy, disomy, or nullisomy) (1,2,14–18); the resulting chromosomal abnormalities in the embryo (polyploidy, aneuploidy) lead to asynchrony and delay of the initial cell cycles of embryo division and, consequently, to a slower embryo development. The incidence of numerical chromosome anomalies in slow or arrested preimplantation embryos is 57–71%, mainly polyploidy (19–21), probably resulting from fertilization of the oocytes by diploid sperm (22), while the figure for well-developed preimplantation embryos is 21–45% (23–25), mainly aneuploidy (19,21,25). This suggests both lower preimplantation embryo viability and a highly effective and early mechanism of embryo chromosomal natural selection related to the severity of chromosome anomalies (21,24–26); this may produce infertility through implantation failure, spontaneous abortion in the first trimester (14,26), or “de novo” fetal aneuploidy (27).

The lower viability in the slow preimplantation embryos (28–31) produced by the above-mentioned sperm subpopulations could also lead to the production of fewer good quality embryos available for transfer (especially in low ovulatory response cycles) and presumably to lower implantation and pregnancy rates (especially in nonselective transfers) (32,33). Further studies on a larger patient sample are required to confirm this relationship. In the present sample, 37 clinical pregnancies were obtained, of which 5 were clinical abortions. The rates of implantation, pregnancy per retrieval cycle, abortion, and live births per retrieval cycle were 23.21%, 49.33%, 13.51%, and 42.66%, respectively. In this sample, there were no significant differences in these rates for the spermatogenic patterns described. It should be pointed out that three patients from the initially studied series had a history of spontaneous abortion in the first trimester, and that in two of these the husband had meiotic anomalies (1,2). The abortion rate in the control group was 8.33%.

In conclusion, although the overall clinical outcomes of ICSI in severe idiopathic oligoasthenozoospermia are good, our data indicate the presence of sperm subpopulations that entail meiotic chromosomal risk (meiotic anomalies, sperm concentration $\leq 1 \times 10^6/\text{mL}$) and lead to a slow early embryo

development. Our study also suggests that the early developmental capacity of the embryo is inversely related to the severity of qualitative/quantitative spermatogenic impairment (meiotic anomalies and/or sperm concentration $\leq 1 \times 10^6/\text{mL}$). Therefore, in ICSI patients with oligoasthenozoospermia and with spermatogenic patterns of meiotic risk, a slow early embryo development is suggestive of a possible chromosomal risk of paternal origin.

These findings support the need for cytogenetic studies (meiosis or/and sperm FISH) in males with a severe oligoasthenozoospermia (1,2) and for genetic counselling and preimplantation diagnosis during the ICSI cycle in the "high-risk" patients.

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