4.1.3 Artículo 3

Título: Meiotic abnormalities and spermatogenic parameters in severe oligoasthenozoospermia.

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Meiotic abnormalities and spermatogenic parameters in severe oligoasthenozoospermia

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The incidence of meiotic abnormalities and their relationship with different spermatogenic parameters was assessed in 103 male patients with presumably idiopathic severe oligoasthenozoospermia (motile sperm concentration $\leq 1.5 \times 10^6$/ml). Meiosis on testicular biopsies was independently evaluated by two observers. Meiotic patterns included normal meiosis and two meiotic abnormalities, i.e. severe arrest and synaptic anomalies. A normal pattern was found in 64 (62.1%), severe arrest in 21 (20.4%) and synaptic anomalies in 18 (17.5%). The overall rate of meiotic abnormalities was 37.9%. Most (66.7%) meiotic abnormalities occurred in patients with a sperm concentration $\leq 1 \times 10^6$/ml. In this group, total meiotic abnormalities were found in 57.8% of the patients; of these, 26.7% had synaptic anomalies. When the sperm concentration was $\leq 0.5 \times 10^6$/ml, synaptic anomalies were detected in 40% of the patients. In patients with increased follicle stimulating hormone (FSH) concentrations, total meiotic abnormalities occurred in 54.8% (synaptic anomalies in 22.6%). There were statistically significant differences among the three meiotic patterns in relation to sperm concentration ($P < 0.001$) and serum FSH concentration ($P < 0.05$). In the multivariate analysis, sperm concentration $\leq 1 \times 10^6$/ml and/or FSH concentration >10 IU/l were the only predictors of meiotic abnormalities.

Key words: intracytoplasmic sperm injection /meiosis/ meiotic chromosome abnormalities/oligoasthenozoospermia/ sperm parameters

Introduction

In couples suffering from severe idiopathic oligoasthenozoospermia, results of assisted fertilization methods prior to the introduction of intracytoplasmic sperm injection (ICSI) were highly discouraging. In 1992, Palermo *et al.* reported the first ongoing pregnancies and deliveries following ICSI. Presently, ICSI is the most efficient assisted fertilization technique in cases of severe male factor infertility (Van Steirteghem *et al.*, 1993a,b; Calderón *et al.*, 1995a; Nagy *et al.*, 1995). Furthermore, it has been shown that testicular sperm extraction plus ICSI allows the development of viable embryos and the establishment of viable pregnancies in apparently azoospermic (obstructive or non-obstructive) men (Schoysman *et al.*, 1993; Devroey *et al.*, 1994, 1995; Silber *et al.*, 1994, 1995; Tournaye *et al.*, 1994; Calderón *et al.*, 1995b, 1997). Thus, genetically normal epididymal, testicular or ejaculated spermatozoa can be used for ICSI.

On the other hand, chromosome abnormalities may be responsible for male infertility. Indeed, the prevalence of somatic chromosome abnormalities detectable in the karyotype is 10 times higher in infertile men (5.3%) than in the general population (0.6%) (Egozcue, 1989). Numerical and structural anomalies of the sex chromosomes occur with a high frequency, mainly in azoospermic and severely oligoasthenozoospermic men (Retief, 1986). In addition, the incidence of synaptic chromosome anomalies restricted to the germ cell line and only detectable by meiotic studies is 4–7.7% in cases of male infertility (Egozcue *et al.*, 1983; De Braekeleer and Dao, 1991).

The aim of this study was to evaluate the relationship between different spermatogenic parameters [testicular size, sperm concentration and motility, baseline serum follicle stimulating hormone (FSH) and count of mature spermatids per tubule] and the presence of meiotic abnormalities (severe arrest and synaptic anomalies) in patients with severe oligoasthenozoospermia and therefore likely to undergo ICSI.

Materials and methods

The population studied consisted of 103 male patients who were consecutively referred to the unit of andrology of our institution because of the detection of extreme oligoasthenozoospermia (motile sperm concentration $\leq 1.5 \times 10^6$/ml), presumably of idiopathic origin, in at least two previous sperm samples. The ICSI protocol was approved by the Ethical Committee of Institut Universitari Dexeus, and written informed consent was obtained from all participants.

In all patients, salient features of clinical history and physical examination were recorded as well as results of the following investigations: testicular volume, semen evaluation, baseline serum FSH concentration, and a testicular biopsy.

Testicular dimensions were measured with calipers. Testicular volume was estimated by the following formula (Ley and Leonard, 1985): $V = 4/3\ p\ (a/2)^2\ (b/2)$, where 'a' equals the short testicular axis, and 'b' equals the long testicular axis (in cm). Volumes are expressed in ml as the average of the two testicles.

Seminal fluid was collected by masturbation after 3–5 days of abstinence and in the absence of fever for 3 months before the study. All samples were analysed for volume (ml). Sperm concentration ($\times 10^6$/ml), motility (%) and motile sperm concentration ($\times 10^6$/ml) were assessed by means of the Makler chamber (Makler, 1978; Makler *et al.*, 1980). Baseline serum FSH was measured by radioimmunoassay

Table I. General characteristics of 103 male patients with severe oligoasthenozoospermia

Data	Mean (SEM)
Age, years	35.13 (0.51)
Length of infertility (years)	4.16 (0.33)
Semen parameters	
Seminal volume (ml)	3.66 (0.17)
Sperm concentration ($\times 10^6$/ml)	2.85 (0.36)
Motility (%)	22.47 (1.74)
Motile sperm concentration ($\times 10^6$/ml)	0.49 (0.07)
Testicular volume (ml)	11.74 (0.52)
Serum FSH concentration (IU/l) (n = 89)	10.17 (0.90)
Count of mature spermatids (n = 71)	5.33 (0.84)

with a commercial kit (Abbott Laboratories, S.A.). A single blood sample for each patient was analysed.

A testicular biopsy was taken under local anaesthesia. The biopsy was performed unilaterally for the study of meiosis according to the method described by Egozcue *et al.* (1983) when a histological diagnosis was already available, and bilaterally for meiotic studies, for histological evaluation (Levin, 1979) and for counting of the number of mature spermatids per 20 tubules (Silber and Rodriguez-Rigau, 1981) when a previous histological diagnosis was not available. Meiosis on testicular biopsy material was independently evaluated by two observers. Three meiotic patterns were defined: normal meiosis and two meiotic abnormalities, i.e. severe arrest (presence of pachytenes and occasional spermatozoa, but no metaphase I figures found) and synaptic anomalies (chromosome pairing anomalies). Peripheral blood karyotypes were also evaluated.

Statistical analysis

The four quantitative parameters were dichotomized as follows: sperm concentration, $\leq 1\times10^6$/ml versus $>1\times10^6$/ml; motile sperm concentration, $\leq 0.5\times10^6$/ml versus $>0.5\times10^6$/ml; testicular volume, <15 ml versus ≥ 15 ml (normal range); and serum FSH concentration, >10 IU/l versus ≤ 10 IU/l (normal range). Meiotic abnormalities (arrest and synaptic anomalies) were analysed together and separately. The Student's t-test and the analysis of variance (ANOVA) were used for the comparison of quantitative variables and the chi-square test (χ^2) for categorical variables. The independent predictive value of significant variables in the univariate analysis was assessed by means of a logistical regression model. All statistical tests were performed at the 5% level of significance. The Statistical Package for the Social Sciences (SPSS) for Windows was used for the analysis of data.

Results

General characteristics of the population studied are shown in Table I. Ages of the patients ranged between 26 and 59 years and time of infertility between 1 and 22 years. The seminal volume varied from 0.8 to 9 ml and sperm concentration between 0.01 and 10.6×10^6/ml. Motile sperm concentration ranged between 0 and 1.5×10^6/ml being less than 1×10^6/ml in 88 (85.4%) of the 103 patients. Testicular volume ranged between 2.75 and 25.8 ml. It was <15 ml in 73 (70.9%) patients. Baseline serum FSH concentrations were determined in 89 patients ranging between 1 and 37.49 IU/l. Increased FSH concentrations (>10 IU/l) were found in 31 (34.8%) patients. Quantitative analysis of testicular biopsy was performed in 71 (68.9%) patients in whom the mean number of mature spermatids per tubule ranged between 0.15 and 21.5.

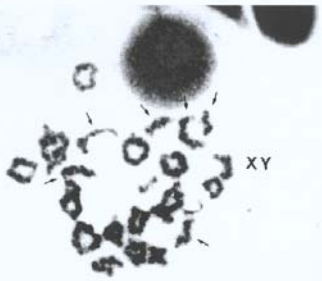

Figure 1. Metaphase I with a severe reduction in the number of chiasmata in several bivalents (arrows). The XY bivalent is indicated (original magnification $\times$3000).

Table II. Relationship between severe oligoasthenozoospermia and meiotic patterns

	Meiotic pattern			
Data	Normal (n = 64)	Arrest (n = 21)	Synaptic anomalies (n = 18)	P value
Age, years	34.84 (0.64)	37.05 (1.38)	33.89 (0.82)	NS
Length of infertility (years)	3.88 (0.35)	5.62 (1.03)	3.44 (0.68)	NS
Seminal volume (ml)	3.66 (0.22)	3.44 (0.34)	3.91 (0.49)	NS
Motility (%)	19.62 (1.71)	25.93 (3.91)	28.61 (6.29)	NS
Count of mature spermatids[a]	5.19 (0.65)	6.24 (3.45)	4.77 (1.19)	NS

Data as mean (SEM).
[a]Number of patients studied: normal pattern, 42; arrest, 15; synaptic anomalies, 14.

Histological diagnosis was incomplete arrest of spermatogenesis in 91.3% of cases and hypo-spermatogenesis in the remaining cases.

A total of 70 men, randomly chosen, was karyotyped and a single case of *46,XY/47,XXY* mosaicism was found. The distribution of meiotic abnormalities in the karyotyped group (normal 62.8%, arrest 18.6%, synaptic anomalies 18.6%) was not significantly different (P = 0.77) from that in patients not karyotyped (normal 60.6%, arrest 24.2%, synaptic anomalies 15.2%).

Meiotic studies in testicular samples were performed in all 103 patients. A normal pattern was found in 64 (62.1%), severe arrest in 21 (20.4%), and synaptic anomalies in 18 (17.5%) (Figure 1). The overall rate of meiotic abnormalities was 37.9%. There were no statistically significant differences among patients with normal or abnormal meiotic patterns in relation to mean age, length of time of infertility, seminal volume, percentage of motile spermatozoa, and number of mature spermatids per tubule (Table II). However, in patients with sperm concentration $\leq 1\times10^6$/ml, motile sperm concentration $\leq 0.5\times10^6$/ml and serum FSH concentrations >10 IU/l, meiotic abnormalities were significantly more frequent than

Table III. Frequency distribution of meiotic patterns in different subgroups of patients

	Meiotic pattern			
Data	Normal (n = 64)	Arrest (n = 21)	Synaptic anomalies (n = 18)	Abnormal[a] (n = 39)
Sperm concentration ($\leq 10^6$/ml) (n = 45)[b,c]	19 (29.7)	14 (66.7)	12 (66.7)	26 (66.7)
Motile concentration ($\leq 0.5 \times 10^6$/ml) (n = 76)[d]	42 (65.6)	18 (85.7)	16 (88.9)	34 (87.2)
Testicular volume (<15 ml) (n = 73)	44 (68.8)	15 (71.4)	14 (77.8)	29 (74.4)
Serum FSH concentration (>10 IU/l) (n = 31)[d,e,f]	14 (25.5)	10 (55.6)	7 (43.8)	17 (50.0)

Data expressed as number of patients and percentage (in parentheses) in relation to meiotic patterns.
[a]Abnormal meiotic pattern: arrest and synaptic anomalies.
[b]$P < 0.001$ as compared with abnormal pattern.
[c]$P = 0.001$ as compared with arrest and synaptic anomalies.
[d]$P < 0.05$ as compared with abnormal pattern.
[e]$P < 0.05$ as compared with arrest and synaptic anomalies.
[f]Number of patients studied: normal pattern, 55; arrest, 18; synaptic anomalies, 16; abnormal pattern, 34.

Table IV. Results of logistic regression analysis. Risk factors for normal or abnormal meiosis

Variable	Coefficient (β)	Standard error	Wald χ^2	d.f.	P value
Sperm concentration	1.3032	0.4734	7.5799	1	0.0059
Serum FSH concentration	–1.0436	0.4858	4.6154	1	0.0317
Constant	–0.4587	0.4442	1.0663	1	0.3018

normal meiotic patterns (Table III). Total meiotic abnormalities and synaptic anomalies accounted respectively for 57.8 and 26.7% of patients with sperm counts $\leq 1 \times 10^6$/ml and for 54.8 and 22.6% of patients with FSH concentrations >10 UI/l. After multivariate analysis, sperm concentration and serum FSH concentration appeared to be the only independent predictive factors of normal or abnormal meiotic patterns (Table IV). Motile sperm concentration was not a predictive factor because there was a statistically significant correlation with sperm concentration ($r = 0.653$; $P < 0.0001$).

Discussion

Although the incidence of somatic chromosome anomalies in infertile male patients with severe oligoasthenozoospermia or azoospermia has been well documented (Retief, 1986; Egozcue, 1989; Reijo *et al.*, 1995), there are few studies on the incidence of meiotic chromosome abnormalities (Egozcue *et al.*, 1983; De Braekeleer and Dao, 1991; Lange *et al.*, 1997), especially in male ICSI candidates (Pieters *et al.*, 1998).

The population studied suffered from severe non-obstructive oligoasthenozoospermia, i.e. low motile sperm concentration (mean 0.49×10^6/ml), moderately hypoplastic testicular volume (mean 11.74 ml); slightly elevated baseline serum FSH level (mean 10.7 IU/l), and low count of mature spermatids per tubule (mean 5.33) and met the criteria for inclusion in an ICSI programme. In this population, we found a high incidence of meiotic abnormalities (37.9%), 17.5% of which corresponded to synaptic anomalies. This incidence is ~2.5 times higher than in heterogeneous male infertility (Egozcue *et al.*, 1983), suggesting that the higher frequency of meiotic chromosome abnormalities may often be responsible for infertility in patients with severe oligoasthenozoospermia.

The present results also demonstrate that most total meiotic abnormalities (66.7%) and synaptic anomalies (66.7%) were found in patients with sperm concentrations $\leq 1 \times 10^6$/ml, affecting 57.8 and 26.7% of the patients respectively. When the sperm concentration was $\leq 0.5 \times 10^6$/ml, 32% of patients had a normal meiotic pattern, 28% had a severe arrest and 40% had synaptic anomalies ($P < 0.001$). The incidence of synaptic anomalies is about five to six times higher than for the general infertile male population. These data suggest a relationship between the incidence of meiotic chromosome abnormalities and the degree of impairment of spermatogenesis. In our opinion, this high incidence of meiotic abnormalities in severe oligoasthenozoospermia makes meiotic studies in male infertility advisable before ICSI if they are available. Meiotic studies can be performed on testicular tissue samples (Egozcue *et al.*, 1983; Lange *et al.*, 1997) or in semen (Sperling and Kaden, 1971; Templado *et al.*, 1980), although in the ejaculate sufficient material for a consistent diagnosis is only obtained in 25–30% of cases.

Meiotic abnormalities include severe meiotic arrest and synaptic anomalies. Severe meiotic arrest due to synaptic anomalies or to unknown causes results in an arrest of spermatogenesis, usually at the stage of primary spermatocyte, resulting in oligozoospermia or azoospermia. Synaptic anomalies limited to the germ cell line in patients with normal karyotype and, therefore, only detectable by meiotic studies, are usually associated with an incomplete meiotic arrest and oligoasthenozoospermia (or azoospermia in case of complete meiotic arrest) and/or subsequent production of chromosomically abnormal spermatozoa (sperm aneuploidies or diploidies), which may be responsible for male infertility, spontaneous abortions during the first trimester of pregnancy or fetal chromosome abnormalities (Egozcue *et al.*, 1983). In the present series, a former history of spontaneous abortions was recorded in three cases (2.9%). All three patients had a normal karyotype but synaptic anomalies were found in two of them. The single patient with an abnormal karyotype (*46,XY/47,XXY*) had a normal meiotic pattern.

In our group of 31 patients with increased FSH levels (>10 IU/l), total meiotic abnormalities occurred in 54.8% and synaptic anomalies in 22.6%. In contrast with others (Novero *et al.*, 1997), this high incidence of meiotic abnormalities leads us to recommend the inclusion of FSH measurement in the routine evaluation of infertile male patients with oligoasthenozoospermia for ICSI.

We conclude that in male infertility due to oligoasthenozoospermia, sperm concentrations $\leq 1 \times 10^6$/ml and/or baseline serum FSH levels >10 IU/l are significant predictors of meiotic abnormalities. In these cases, meiotic chromosome studies can be performed to identify and characterize cytogenetic errors. This would allow the characterization of a particular 'high-risk'

group that needs genetic counselling and prenatal diagnosis in case of establishment of a pregnancy in ICSI cycles.

Meiotic studies are predictive, but they not provide information on the final status of the gametes. On the other hand, fluorescent in-situ hybridization (FISH) on decondensed sperm heads only provides information on the status of the chromosome pairs analysed with the probes used, may be difficult to perform on a significant number of spermatozoa in cases with very low sperm counts. If possible, we would recommend sperm chromosome studies, although they take a long time and are expensive, which makes them unpractical in clinical work.

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4.1.4 Artículo 4

Título: Spermatogenic patterns and early embryo development after intracytoplasmic sperm injection in severe oligoasthenozoospermia.

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Spermatogenic patterns and early embryo development after intracytoplasmic sperm injection in severe oligoasthenozoospermia

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Running title: Sperm patterns and embryo development

This controlled study suggests that the early embryonic developmental capacity is inversely related to the severity of spermatogenic impairment (meiotic anomalies and/or sperm concentration $\leq 1x10^6$/ml).

Purpose: Evaluate the influence of different baseline spermatogenic patterns (meiotic pattern (normal or abnormal), sperm concentration (>1x10^6/ml or ≤1x10^6/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 oligoasthenozoospermic 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 oligoasthenozoospermic group for meiotic anomalies (43.40%), sperm concentration ≤1x10^6/ml (44.35%) and the combined pattern ≤1x10^6/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 ≤1x10^6/ml (OR 1.53; 95%CI 1.09-2.13)) were present.

Conclusions: The data suggests that the early embryonic developmental capacity is inversely related to the severity of spermatogenic impairment (meiotic anomalies and/or sperm concentration ≤1x10^6/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 non-obstructive 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 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 $\leq 1x10^6$/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 ($>1x10^6$/ml or $\leq 1x10^6$/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 non-obstructive 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 asssessed 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 $\leq 1.5x10^6$/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 suppresion is started in the mid-luteal 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 eighteen hours after sperm microinjection, oocytes were observed to check for fertilization (2PN) and, 24 hours 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.

Dataset and statistical analysis

In the oligoasthenozoospermic group, the independent spermatogenic variables were dichotomized as follows: meiotic pattern (normal (N) or meiotic anomalies (AN)) and sperm concentration ($>1x10^6$/ml or $\leq 1x10^6$/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 ($>1x10^6$N, $\leq 1x10^6$N, $>1x10^6$AN, $\leq 1x10^6$AN).

The dependent embryonic variables were also defined as dichotomies: fertilization (present or absent), zygote division (present or absent) and embryo division rate ($\geq$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 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 seventy-five couples studied and the control group couples are shown in Table I.

In the oligoasthenozoopermic patients group, sperm concentration ranged between $0.01x10^6$/ml and $10.6x10^6$/ml. In 34 (45.33%) of patients it was $\leq 1x10^6$/ml. Motility ranged between 0 and 40.13% and motile sperm concentration ranged between 0 and $\leq 1.5x10^6$/ml.

Frequency distribution for the meiotic pattern was: 53 (70.67%) 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 metapahase I figures).

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

In the control group, sperm concentration ranged between $22.5x10^6$/ml and $188.5x10^6$/ml. The age of patients ranged from 25 to 48 and the period of infertility

between 2 and 18 years. The age of the women ranged between 27 and 40 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%, $p<0.01$).

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 $>1x10^6$/ml and the combined pattern $>1x10^6$/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 $\leq 1x10^6$/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 $\leq 1x10^6$/ml, and a synergic effect was observed (OR 2.00; 95% CI 1.28-3.12) when both risk patterns were combined. The multivariant 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 commbined 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 anomalies (38.1%) in this population (1) which increased (57.8% when the sperm concentration was $\leq 1x10^6$/ml and 68% when it was $\leq 0.5x10^6$/ml) with the severity of spermatogenic impairment (2). In our opinion the baseline study not only provides etiopathogenic information, but avoids both physiological intra-individual variability in the semen analysis (5) as well as 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 $\leq 1x10^6$/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 $\leq 1x10^6$/ml (55.65%, $p<0.05$) and, especially, the combined pattern, i.e., $\leq 1x10^6$/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 $\leq 1x10^6$/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 pre-implantation 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 pre-implantation embryos is 21-45% (23-25), mainly aneuploidy (19,21,25). This suggests both lower pre-implantation 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 sub-populations 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 non-selective transfers) (32-33). Further studies on a larger patient sample are required to confirm this relationship. In the present sample, thirty seven clinical pregnancies were obtained, of which five 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 sub-populations which entail meiotic chromosomal risk (meiotic anomalies, sperm concentration $\leq 1x10^6$/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 $\leq 1x10^6$/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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Table I. General characteristics of the patients

	Normozoospermia	Oligoasthenozoospermia	p
n	79	75	
Man's age (years)[a]	35.84 (0.48)	34.91 (0.55)	NS[b]
Length of infertility (years)[a]	5.07 (0.39)	4.48 (0.43)	NS[b]
Sperm concentration ($x10^6$/ml)[a]	79.95 (6.98)	2.91 (0.46)	<0.001[b]
Woman's age (years)[a]	34.04 (0.37)	32.93 (0.44)	NS[b]
Oocytes inseminated[a]	10.76 (0.66)	10.79 (0.65)	NS[b]

[a]Mean (SEM).
[b]Student's t-test.

Table II. Overall embryo outcomes (%)

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

[a]Chi-square test.

Table III. Embryo outcomes (%) according to spermatogenic patterns.

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

[a]Chi-square test.

Table IV. Embryo outcomes (%) according to the combined meiosis-sperm concentration pattern.

	Combined pattern				
	>1x10^{6}N	≤1x10^{6}N	>1x10^{6}AN	≤1x10^{6}AN	p
Oocytes microinjected	388	191	64	166	
Oocytes fertilized	290(74.74)	126(65.96)	46(71.87)	113(68.07)	NS[a]
Cleaved embryos	278(95.86)	109(86.51)	45(97.83)	106(93.81)	NS[a]
Embryos ≥4 cells	157(54.14)	64(50.79)	27(58.69)	42(37.17)	<0.01[a]

N = Normal meiosis, AN = Meiotic anomalies.
[a]Chi-square for trend.

4.2 RESUMEN DE LOS RESULTADOS

La Tabla I (pág.84) muestra la distribución de frecuencias de los patrones espermatogénicos dicotómicos según los tres patrones meióticos estudiados (normal, bloqueo (B), anomalías sinápticas (S)) y también según el patrón anomalías meióticas (B+S). Existe significación estadística respecto a concentración espermática ($p<0.001$) y FSH ($p<0.05$) tanto entre los patrones meióticos normal y anomalías meióticas como entre los tres patrones meióticos básicos aisladamente. Respecto a la concentración espermática mótil existe significación estadística entre los patrones meióticos normal y anormal ($p<0.05$), pero no al considerar los tres patrones aisladamente. No existen diferencias significativas respecto al volumen testicular.

El análisis de regresión logística multivariante (Tabla II, pág. 85) evidenció que los dos únicos factores predictivos independientes respecto a los patrones meióticos dicotómicos normal y anomalías meióticas son la concentración espermática ($p<0.01$) y la concentración sérica basal de FSH ($p<0.05$). La concentración espermática mótil debe considerarse como una variable dependiente de la concentración espermática.

La Tabla III (pág. 86) muestra los resultados embrionarios tras ICSI según los patrones espermatogénicos dicotómicos. Respecto al patrón meiótico existe un ritmo de división bajo (43.40%, $p<0.05$) para anomalías meióticas, no existiendo diferencias en las tasas de fecundación y división. Respecto a la concentración espermática, existen menores tasas de fecundación ($p<0.05$) y división embrionaria ($p<0.01$) y un ritmo de división bajo (44.35%, $p<0.05$) para concentración espermática $\leq 1x10^6$/ml. Respecto a la concentración sérica de FSH no se hallaron diferencias estadísticamente significativas en las tasas de fecundación y división ni en el ritmo de división embrionaria.

La Tabla IV (pág. 87) muestra los resultados embrionarios para el patrón combinado meiosis-concentración espermática resultante de dichas variables significativas. No existen diferencias en las tasas de fecundación y división entre los cuatro patrones resultantes, existiendo una tendencia significativa a enlentecer el ritmo de división embrionaria en relación a la severidad del patrón espermatogénico (54.44% vs 37.17%, $p<0.01$).

La Tabla V (pág. 88) muestra el valor predictivo de los patrones espermatogénicos significativos respecto al ritmo de división embrionaria. El riesgo relativo de desarrollo embrionario temprano lento para anomalías meióticas o concentración espermática $\leq 1x10^6$/ml es 1.5 ($p<0.05$), existiendo un efecto aditivo (2.00, $p<0.01$) cuando coexisten ambos patrones predictivos de riesgo.

Tabla I. Distribución de frecuencias (%) de los patrones meióticos según patrones espermatogénicos

	Meiosis			
	N (n=64)	B (n=21)	S (n=18)	B+S (n=39)
Concentración espermática ($\leq 10^6$/ml)(n=45)[a,b]	19 (29.7)	14 (66.7)	12 (66.7)	26 (66.7)
Concentración espermática mótil($\leq 0{,}5x10^6$/ml)[c](n=76)	42 (65.6)	18 (85.7)	16 (88.9)	34 (87.2)
Volumen testicular (< 15 ml)(n=73)	44 (68.8)	15 (71.4)	14 (77.8)	29 (74.4)
FSH (>10 UI/l)[c,d,e] (n=31)	14 (25.5)	10 (55.6)	7 (43.8)	17 (50.0)

N= Meiosis normal, B= Bloqueo meiótico, S= Anomalías sinápticas, B+S= Anomalías meióticas.

[a] $p< 0.001$ respecto de B+S
[b] $p= 0.001$ respecto de B y S
[c] $p< 0.05$ respecto de B+S
[d] $p< 0.05$ respecto de B y S

[e] Número de pacientes estudiados: Meiosis normal, 55; Bloqueo meiótico, 18; Anomalías sinápticas, 16; Anomalias meióticas, 34.

Tabla II. Regresión logística multivariante. Factores de riesgo para meiosis normal o anomalías meióticas

Variables incluídas en la ecuación de regresión	β	SE	Wald	gl	p
Concentración espermática	1,3032	0,4734	7,5799	1	0,0059
FSH	-1,0436	0,4858	4,6154	1	0,0317
Constante	-0,4587	0,4442	1,0663	1	0,3018

β = Coeficiente, SE = Error estándar.

Tabla III. Resultados embrionarios (%) según patrones espermatogénicos

	Patrón meiótico			Concentración espermática			FSH sérica (n=65)		
	Normal	Anormal	p	$>10^6$/ml	$\leq 10^6$/ml	p	≤10UI/l	>10UI/l	p
Ovocitos microinyectados	579	230		452	357		468	242	
Ovocitos fecundados	416(71.85)	159(69.13)	NS[a]	336(74.34)	239(66.95)	<0.05[a]	336(71.79)	167(69.01)	NS[a]
Embriones divididos	387(93.03)	151(94.97)	NS[a]	323(96.13)	215(89.96)	<0.01[a]	312(92.86)	156(93.41)	NS[a]
Embriones ≥4 células	221(53.12)	69(43.40)	<0.05[a]	184(54.76)	106(44.35)	<0.05[a]	181(53.87)	89(53.29)	NS[a]

[a]Chi-cuadrado test.

Tabla IV. Resultados embrionarios (%) según patrón combinado meiosis-concentración espermática

	Patrón combinado				
	>1x10^{6}N	≤1x10^{6}N	>1x10^{6}AN	≤1x10^{6}AN	p
Ovocitos microinyectados	388	191	64	166	
Ovocitos fecundados	290(74.74)	126(65.96)	46(71.87)	113(68.07)	NS[a]
Embriones divididos	278(95.86)	109(86.51)	45(97.83)	106(93.81)	NS[a]
Embriones ≥4 células	157(54.14)	64(50.79)	27(58.69)	42(37.17)	<0.01[a]

N = Meiosis normal, AN = Anomalías meióticas.
[a]Chi-cuadrado para tendencia.

Tabla V. Regresión logística. Riesgo relativo de desarrollo embrionario temprano lento

Patrón espermatogénico	OR (95% IC)	SE	p
Anomalías meióticas	1.49(1.03-2.15)	0.28	0.035
$\leq 1x10^6$/ml	1.53(1.09-2.13)	0.26	0.013
$\leq 1x10^6$AN	2.00(1.28-3.12)	0.46	0.002

AN= Anomalías meióticas.
OR= Odds ratio, IC= Intervalo de confianza, SE= Error estándar.

5. DISCUSIÓN

La población estudiada es consistente con un diagnóstico de oligoastenozoospermia secretora severa (baja concentración espermática mótil (0.49 x 10^6 /ml), volumen testicular promedio moderadamente hipoplásico (11.74 ml), concentración sérica basal de FSH discretamente elevada (10.7 UI/l) y contaje promedio de espermátides maduras bajo (5.33)), cumpliendo además con los criterios habitualmente considerados como indicación de ICSI.

En dicha población los resultados del estudio espermatogénico constatan una elevada incidencia de anomalías meióticas (37.9%), y especialmente de anomalías sinápticas (17.5%), representando ésta un incremento de aproximadamente 2.5 veces la incidencia de la población general masculina estéril (Egozcue et al 1983), indicando la posible existencia de una mayor frecuencia de etiología genética meiótica en la oligoastenozoospermia severa. Los resultados reflejan asimismo que la mayoría de anomalías meióticas (66.7 %) y anomalías sinápticas (66.7 %) se acumulan en concentraciones espermáticas $\leq 1x10^6$ /ml, afectando respectivamente al 57.8% y 26.7% de pacientes (p=0.001), y alcanzando respectivamente un 68% y un 40% (incidencia aproximadamente 5-6 veces mayor que la de la población general masculina estéril) de pacientes cuando la concentración espermática es $\leq$ 0.5 x 10^6/ml ($p<0.001$). Los datos sugieren un aumento de la incidencia de anomalías meióticas relacionado con la severidad de la alteración de la espermatogénesis.

En nuestro criterio, esta alta incidencia de anomalías meióticas en oligoastenozoospermias secretoras severas hace aconsejable ofrecer un estudio de meiosis a dichos pacientes dentro del estudio andrológico previo a su posible inclusión en un programa de ICSI. El estudio de la meiosis permite analizar el comportamiento cromosómico meiótico para detectar o descartar un comportamiento meiótico anormal.

En la población estudiada asimismo se constató que la mitad de las anomalías meióticas cursan con FSH > 10 UI/l, afectando al 50.8% de pacientes con FSH elevada y hallándose anomalías sinápticas en el 22.6% de dichos pacientes ($p<0.05$), y además que esta significación no es dependiente de la concentración espermática.

En nuestro criterio, en oligoastenozoospermias severas ello es un motivo para incluir la determinación de FSH en el estudio andrológico previo a ICSI, frente a otros autores que la desaconsejan (Novero et al 1997), por cuanto puede ser considerada como un factor predictivo de posible riesgo genético meiótico.

Existen escasos estudios analizando los resultados embrionarios tras ICSI en oligoastenozoospermia según parámetros espermatogénicos basales aislados (Novero et al 1997) y ninguno según el patrón meiótico. Otros estudios analizan los resultados según parámetros espermáticos cuantitativos en la muestra seminal de ICSI (Nagy et al 1995).

Nuestro estudio analiza los resultados embrionarios obtenidos en un primer ciclo de ICSI según distintos patrones espermatogénicos basales (patrón meiótico, concentración espermática, patrón combinado meiosis-concentración espermática, FSH sérica). Se incluyeron los 75 pacientes de la población estudiada que realizaron dicho ciclo en el transcurso del año siguiente a su consulta. En nuestra opinión el estudio basal además de aportar información etiopatogénica, permite obviar tanto la variabilidad intraindividual fisiológica del seminograma (WHO 1999) como el posible sesgo de la muestra seminal de ICSI debido al estrés del ciclo (Clarke et al 1999).

Globalmente las tasas de fecundación (71.08%) y de división de los zigotos (93.57%) fueron normales y el ritmo de división en día 2 (50.43%) lento ($p<0.01$), como corresponde a varones con oligoastenozoospermia severa (Janny et al 1994). Todos los grupos fueron comparables respecto a edad de la mujer y número de ovocitos microinyectados por ciclo (factor ovulatorio normal), por lo que las diferencias halladas en los resultados embrionarios podrían atribuirse a las distintas subpoblaciones espermáticas.

Respecto a la tasa de fecundación únicamente se halló una menor tasa para concentración espermática $\leq 1x10^6$/ml ($p<0.05$). Otros autores (Nagy et al 1995) hallan una menor tasa de fecundación ($p<0.001$) para contajes espermáticos totales muy bajos en muestra seminal de ICSI, no hallándose diferencias según el nivel de FSH sérica (Novero et al 1997).

Respecto al ritmo de división embrionaria se constató un ritmo significativamente bajo en día 2 con predominio de embriones lentos para anomalías meióticas (56.60%, $p<0.05$), para concentración espermática $\leq 1x10^6$/ml (55.65%, $p<0.05$) y especialmente para el patrón combinado $\leq 1x10^6$/ml con anomalías meióticas (62.83%, $p<0.01$), existiendo una tendencia significativa a enlentecer el ritmo de división embrionaria relacionada con la severidad de la alteración de la espermatogénesis y evidenciándose un efecto aditivo (OR 2.00; 95% IC 1.28-3.12) cuando coexisten los dos patrones espermatogénicos predictivos de desarrollo embrionario temprano lento (anomalías meióticas (OR 1.49; 95% IC 1.03-2.15) y concentración espermática $\leq 1x10^6$/ml (OR 1.53; 95% IC 1.09-2.13)).

Se ha sugerido una importante contribución paterna en el desarrollo embrionario temprano, constatándose que una oligoastenozoospermia severa comporta una menor capacidad embriogénica (Janny et al 1994, Ménézo et al 1995) resultando en una división embrionaria temprana lenta tendente a ulterior detención embrionaria (Alikani et al 2000) generalmente durante la activación genómica (Bolton et al 1989, Janny et al 1994), y consiguente pérdida embrionaria preimplantatoria.

Por otra parte se conoce que las anomalías cromosómicas meióticas espermáticas cursan generalmente con oligoastenozoospermia y producción de espermatozoides con una elevada tasa de anomalías cromosómicas numéricas (diploidía, disomías o nulisomías) (Egozcue et al 1983, Martin 1996, Arán et al 1999, Pang et al 1999, Vegetti et al 2000) que generarán embriones cromosómicamente anormales (poliploidía, aneuploidías) (Macas et al 2001, Egozcue et al 2002), condicionando asincronía y retardos en los ciclos celulares iniciales de división embrionaria y consiguientemente una embriogénesis lenta. Es conocido que la incidencia de anomalías cromosómicas numéricas es de un 57-71% en embriones preimplantatorios lentos o detenidos, presentando mayormente poliploidía (Munné et al 1995, Benkhalifa et al 1996, Munné et al 1998) y de un 21-45% en embriones evolutivos preimplantatorios (Plachot et al 1987, Plachot et al 1988, Sandalinas et al 2001) presentando mayormente aneuploidía (Munné et al 1995, Munné et al 1998, Sandalinas et al 2001), sugiriendo tanto una menor viabilidad embrionaria preimplantatoria como una altamente eficaz y temprana selección natural cromosómica embrionaria relacionadas con la severidad de las anomalías cromosómicas (Plachot et al 1988, Munné et al 1998, Egozcue et al 2000), pudiendo clínicamente condicionar esterilidad por fallo implantatorio (Egozcue et al 2002), aborto espontáneo de primer trimestre (Egozcue et al 1983, Egozcue et al 2000) o aneuploidías fetales “de novo” (Bonduelle et al 1998).

De otro lado la menor viabilidad de los embriones preimplantatorios lentos (Giorgetti et al 1995, Ziebe et al 1997, Shoukir et al 1997, Sakkas et al 1998) producidos por dichas subpoblaciones espermáticas (anomalías meióticas, concentración $\leq 1x10^6$/ml) podría también condicionar una menor disponibilidad de embriones transferibles de buena calidad (especialmente en ciclos con baja respuesta ovulatoria) y presumiblemente un pronóstico de inferiores tasas de implantación y gestación (especialmente en tranferencias no selectivas) (Templeton et al 1998, Murdoch 1998), requiriéndose posteriores estudios en una mayor población muestral de pacientes a tal objeto. En la muestra estudiada se obtuvieron globalmente 37 gestaciones clínicas, de las cuales cinco fueron abortos clínicos. Las tasas de implantación, gestación por ciclo y aborto fueron respectivamente 23.21%, 49.33% y 13.51%. Para los patrones espermatogénicos descritos no existieron diferencias estadísticamente significativas en las tasas de implantación, gestación por ciclo y aborto en esta muestra. Cabe señalar que en tres pacientes de la serie inicialmente estudiada existía el antecedente clínico de aborto espontáneo de primer trimestre, presentando dos de ellos anomalías meióticas (Vendrell et al 1999).

Así pues, aunque los resultados clínicos globales tras ICSI en oligoastenozoospermia secretora severa sean buenos, nuestros resultados embrionarios indican la existencia de subpoblaciones espermáticas de riesgo cromosómico meiótico (anomalías meióticas, concentración espermática $\leq 1x10^6$/ml) que condicionan una embriogénesis temprana lenta, sugiriendo una capacidad embriogénica temprana inversamente relacionada con la severidad de la alteración espermatogénica cualitativa/cuantitativa (anomalías meióticas y/o concentración espermática $\leq 1x10^6$/ml). Asimismo una embriogénesis temprana lenta es sugestiva de posible riesgo cromosómico embrionario de origen paternal en

pacientes oligoastenozoospérmicos con anomalías meióticas o/y concentración espermática $\leq 1x10^6$/ml.

En nuestro criterio, el estudio espermatogénico basal posibilita la detección de un subgrupo de pacientes de "alto riesgo genético" que requiere consejo genético y diagnóstico preimplantacional durante el ciclo de ICSI y diagnóstico prenatal citogenético en caso de gestación.

6. CONCLUSIONES

1) Existe una elevada incidencia de anomalías meióticas (37.9 %) y especialmente de anomalías sinápticas (17.5 %) en pacientes con oligoastenozoospermia secretora severa (concentración espermática mótil $\leq 1.5x10^6$/ml).

2) La mayoría (66.7 %) de anomalías meióticas y de anomalías sinápticas se acumulan en pacientes con concentración espermática $\leq 1x10^6$/ml, afectando respectivamente al 57.8 % y 26.7 % de pacientes y alcanzando un 68 % y un 40% respectivamente cuando la concentración espermática es $\leq 0.5x10^6$/ml, sugiriendo un incremento de anomalías meióticas y anomalías sinápticas relacionado con la severidad de la alteración espermatogénica.

3) En pacientes afectos de oligoastenozoospermia secretora severa la existencia de una concentración espermática $\leq 1x10^6$/ml ($p<0.01$) o/y una concentración de FSH sérica basal elevada ($p<0.05$) son patrones espermatogénicos independientes predictivos de riesgo de anomalías meióticas.

4) En pacientes con oligoastenozoospermia secretora severa las tasas de fecundación (71.08 %) y de división de los zigotos (93.57 %) tras ICSI son normales y el ritmo de división embrionaria (≥ 4 células) en día 2 es bajo (50.43 %, $p<0.01$).

5) No existen diferencias en las tasas de fecundación y división según los distintos patrones espermatogénicos, excepto únicamente una menor tasa para concentración espermática $\leq 1x10^6$/ml.

6) Se constata un ritmo de división embrionaria en día 2 (≥ 4 células) bajo para anomalías meióticas ($p<0.05$), para concentración espermática $\leq 1x10^6$/ml ($p<0.05$) y especialmente para el patrón combinado $\leq 1x10^6$/ml con anomalías meióticas ($p<0.01$), existiendo una tendencia a enlentecer el ritmo de división embrionaria relacionada con la severidad de la alteración espermatogénica ($p<0.01$).

7) Existe un efecto sinérgico (OR 2.00; 95 %IC 1.28-3.12) cuando coexisten los dos patrones espermatogénicos predictivos de embriogénesis temprana lenta (anomalías meióticas (OR 1.49; 95 %IC 1.03-2.15) y concentración espermática $\leq 1x10^6$/ml (OR 1.53; 95 %IC 1.09-2.13)).

8) Existen subpoblaciones espermáticas (anomalías meióticas, concentración espermática $\leq 1x10^6$/ml) que condicionan una embriogénesis temprana lenta con predominio de embriones lentos (56.60 % para anomalías meióticas, 55.65 % para concentración espermática $\leq 1x10^6$/ml y 62.83 % para el patrón combinado de ambos).

9) Los datos embrionarios reflejan una capacidad embriogénica temprana inversamente relacionada con la severidad de la alteración espermatogénica cualitativa/cuantitativa (anomalías meióticas y/o concentración espermática $\leq 1x10^6$/ml).

10) Los datos embrionarios sugieren una muy temprana selección natural embrionaria, relacionada con la severidad de la alteración espermatogénica.

11) Una embriogénesis temprana lenta es sugestiva de posible riesgo cromosómico de origen paternal en pacientes oligoastenozoospérmicos con anomalías meióticas o/y concentración espermática $\leq 1x10^6$/ml.

12) Los resultados sugieren que en pacientes con oligoastenozoospermia secretora severa con criterios predictivos de riesgo de anomalías meióticas (concentración espermática $\leq 1x10^6$/ml, concentración de FSH sérica basal elevada) es aconsejable ofrecer un estudio citogenético de la línea germinal en el cribado andrológico previo a ICSI, posibilitando así la detección de un subgrupo de pacientes de "alto riesgo genético" que requiere consejo genético y diagnóstico preimplantacional durante el ciclo de ICSI.

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