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Very Low Sperm Count Affects the Result of Intracytoplasmic Sperm Injection

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Purpose: *The aim was to examine the influence of extremely low sperm count on intracytoplasmic sperm injection (ICSI) outcome.*
Methods: *Over 1000 consecutive unselected ICSI cycles were divided into four groups according to sperm concentration of their patients: A, cryptozoospermia, 107 patients; B, sperm concentration of* $\leq 1 \times 10^4$*, 146 patients; C, sperm count of* 1×10^4*–*1×10^5*, 135 patients; and concentration of* $>1 \times 10^5$ *and* $< 10 \times 10^6$*/ml (control group), 688 patients.*
Results: *A significant decrease in pregnancy rate was noticed in the cryptozoospermic group in comparison to the control group (20% vs. 31%). Fertilization rate in group A was significantly lower in comparison to all other groups, respectively (46% vs. 52%, 54%, 61%). Embryo quality was inferior in group A in comparison to the control group. A higher yet not statistically significant abortion rate was observed in the cryptozoospermic group (as well as in group C) (30%, 27%) compared to the control group (15%).*
Conclusions: *It seems that an extremely low sperm count has a negative effect on the outcome of ICSI. Nevertheless patients with cryptozoospermia should not be offered ICSI treatment with the ejaculated sperm before karyotype is established.*

KEY WORDS: Intracytoplasmic sperm injection; fertilization rate; sperm count; cryptozoospermia.

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) is a successful treatment for severe male subfertility (1) enabling fertilization with the use of ejaculated semen even with very poor conventional sperm parameters. Fertilization may be achieved with ICSI, independent of sperm concentration, motility, or morphology using only one spermatozoon per oocyte. One central question concerning ICSI is whether the type or the extent of sperm impairment has any influence on outcome. A number of investigators examined the effect of different sperm parameters on fertilization and pregnancy rates after ICSI. However, the results are inconsistent. ICSI results were shown to be the same with the various sperm defects like severe oligospermia, oligoasthenoteratospermia, and asthenoteratospermia (2,3). More than that, severe morphological defects of spermatozoa were not shown to impact the fertilization process in ICSI (4). On the other hand, a direct correlation between extensive teratozoospermia of 100% abnormal sperm head and impaired implantation and ongoing pregnancy rates were observed (5). The fertilizability of totally immotile ejaculated spermatozoa was significantly lower when compared with the fertility capacity of initially immotile but further motile sperm (6). The relation between extreme oligozoospermia and ICSI outcome has not been systematically investigated.

The aim of this study was to systematically investigate the effect of extremely low sperm concentration on the outcome following the ICSI procedure.

MATERIALS AND METHODS

Male Patients and Sperm Preparation

The use of ICSI in our program is restricted to patients with sperm concentration of $< 5 \times 10^6$, sperm motility of $<$10%, or normal sperm morphology of $<$10%. At least two out of three mentioned parameters must be fulfilled. An additional indication for ICSI is fertilization of less than 20% of the oocytes in a previous in vitro fertilization (IVF) cycle.

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The study group included 1076 (during the years 1996–1998) consecutive unselected ICSI cycles, which were analyzed retrospectively. The patients were divided into four groups, according to increasing sperm concentration in the ejaculate. In all cases, the evaluation for the presence of sperm was done with × 100 magnification. (a) Group 1 comprised 107 (cryptozoospermic group) cases where no visible spermatozoa were recognized before or after centrifugation over the entire slide (Makler chamber, Sefi Instruments, Haifa, Israel). Sperm cells were discovered only after careful search in dozens of droplets by the extended sperm preparation (ESP) method (7). (b) Group 2 comprised 146 cases with no visible spermatozoa by microscopy before centrifugation, and not more than one motile spermatozoon was visible over the entire slide after centrifugation. This situation correlates approximately with a sperm concentration of less than $\leq 1 \times 10^4$ cell/ml. (c) Group 3 comprised 135 cases in which not more than one spermatozoon was visible by microscopy before centrifugation. This situation correlates approximately with a sperm concentration of between 1×10^4 cell/ml and 1×10^5 cell/ml. (d) Group 4 comprised 688 cases serving as a control group. The patients' sperm count after preparation was between $1 \times 10^5 - 1 \times 10^6$ cell/ml. All patients with severe oligozoospermia (sperm concentration of $< 2 \times 10^6$ cell/ml) had peripheral leukocyte karyotype analysis done according to Wilkins (8). All patients in groups A, B, and C had a normal XY karyotype.

After liquefaction of freshly ejaculated semen, sperm concentration was confirmed using a Makler chamber. Semen was treated by washing twice in Earl's medium (1800 *g* for 5 min) if concentration was less than 0.5×10^6 cell/ml. If sperm concentration was more or equal to 0.5×10^6 cell/ml, sperm was put on three-layer Percoll gradient (Irvine Scientific, Santa Ana, CA) (90%, 70%, and 40%) or two layers of isolate (Irvine Scientific, Santa Ana, CA), centrifuged at 300g for 20 min, and the final pellet washed and centrifuged at 1800 *g* for 5 min. Sperm density was checked and documented after the preparation procedure. Sperm motility was evaluated according to World Health Organization (WHO) recommendations and morphology was determined following eosin-nigrosin staining (9).

Extended Sperm Preparation (ESP)

ESP was performed on the ejaculate in all cases with nonobstructive azoospermia, consisting of a meticulous search for sperm cells as a standard procedure prior to any surgical intervention. The details of the procedure have been described previously (7). Briefly, the semen was washed twice by centrifugation at 350 g for 20 min. Aliquots of 4–6 μl were aspirated from the pellet and distributed in several dozen droplets of 8 μl medium under paraffin oil (embryo tested, Sigma, USA).

Once a motile spermatozoon with acceptable morphology was visible under the inverted microscope, the spermatozoon was aspirated using a collection pipette with an outer diameter of 12 μm and an inner diameter of 10 μm (Perry, Raanana, Israel) and put in the central droplet with polyvinylpyrrolidone (PVP) (Irvine Scientific, Santa Ana, CA) for injecting into the oocyte.

Oocyte Preparation

Controlled ovarian hyperstimulation (COH) and luteal supplementation were performed according to our previous description (10). Female patients were normogonadotrophic, aged (mean ± SD) 32.1 ± 6.2, 33.2 ± 5.7, 33.5 ± 5.4, and 32.5 ± 5.6 in the four groups, respectively. There were no statistically significant differences among the ages of the patients in the different groups. All patients had a normal response to COH. Oocyte retrieval was performed by ultrasound guided puncture. The cumulus–corona cells were removed after exposure to hyaluronidase (Hyaluronidase, type IV-S, Sigma, USA) 20 IU/ml in human tubal fluid medium (HTF) (Irvine Scientific) for no more than a minute. Only metaphase II oocytes were taken for ICSI.

ICSI Procedure

The procedure was carried out according to the methodology described by Van Steirteghem et al. (1). A motile spermatozoon with normal appearance, whenever possible, was selected and injected. Culture of the injected oocytes took place in 25-μl droplets of medium (Medium Cult, Copenhagen, Denmark) under lightweight paraffin oil (Sigma, USA).

Oocytes were observed for fertilization 16–18 hr after micromanipulation. The criteria for normal fertilization were the presence of two polar bodies together with two pronuclei. Cleavage was assessed 24 hr later and the embryos were classified according to their morphological appearance: Embryos classified as excellent—symmetrical blastomeres with or without 20% of the volume filled with anucleated fragments; embryos classified as fair—with 20 to 50% of the volume covered with fragments; and embryos classi-

fied as bad—extreme asymmetrical blastomeres and >50% of the volume filled with anucleated fragments.

Excellent, good, and fair embryos were selected for transfer to the uterine cavity 48–72 hr after the ICSI procedure. Pregnancy rate was calculated considering clinical pregnancies only, determined by the presence of at least one gestational sac by tranvaginal ultrasound 4 weeks after embryo transfer.

Statistical Analysis

Statistical evaluation was performed using student's *t* test, χ^2 test, and Fischer's exact test, where appropriate. A P value of <0.05 was considered statistically significant.

RESULTS

The number of treated cycles, sperm motility, and morphology Kruger criteria in the different study groups are shown in Table I. Sperm motility varied between 64 ± 46.8% and 43 ± 37.5% (mean ± SD) among the groups A to D; sperm morphology varied between 6 ± 6.3% and 8 ± 7.8%. These differences were not statistically significant between the different groups. Sperm evaluation for motility and morphology was not feasible in the 107 cases with cryptozoospermia (group A) since not enough sperm cells were found in order to enable it.

In group A, 1116 out of 1428 oocytes were injected (78%). Fifty matured oocytes of nine patients were not injected since not enough sperm were available for injection. Altogether 1198, 1377, and 6466 MII oocytes were injected in the study groups B to D, respectively, which are about 80% of the retrieved cohort (1548, 1751, and 8101). The average damage rate to the oocytes after the ICSI was 9%. In group A, 10 ± 5.2 MII oocytes were injected per cycle, in group B 10 ± 6.6 MII oocytes, 10 ± 6.8 in group C, and 9 ± 6.4 in group D (Table II). The differences were not statistically significant.

The fertilization rate in the cryptozoospermic group (A) was significantly lower than in groups B, C, and D (46% vs .54%, 46%, 61%), while group B had a significantly lower fertilization rate than group D (Table II). Group D, however, had a slight lower fertilization rate than the average fertilization rate in our general ICSI program, which is 65%. The mean incidence of multiple pronuclei (3PN) was 4% and for the one pronucleus 5%, with no statistically significant difference between the groups.

The cleavage rate in the cryptozoospermia group (A) was slightly lower but the difference was not statistically significant from the average in the three other groups (Table III). The mean number of the embryos per transfer and their mean number of blastomeres were comparable in all groups. The embryos classified as those with excellent morphology were significantly less frequent in group A in comparison with their incidence in groups C and D. These results correlated with the results of four-cell embryos, which were less often observed in group A in comparison to groups B, C, and D. Significantly less freeze quality embryos were obtained after using spermatozoa from group A than using spermatozoa from groups B, C, or D. Pregnancy rate per embryo transfer in group A was significantly lower than in the control group (Table IV), while implantation rate was slightly lower in group A in comparison to the average implantation rate in the three other groups (10% vs. 13%). The first trimester abortion rate was significantly higher in group A than that in the control group, but not significantly higher than in groups B and C.

DISCUSSION

Intracytoplasmic sperm injection enables fertilization using ejaculated sperm samples with very poor conventional sperm parameters, since the only absolute

Table I. Sperm Parameter in the Different Study Groups

Group	Sperm concentration	No. of cases	Motility (%)[a]	Morphology (%±)
A	Cryptozoospermia	107	ND	ND
B	$\leq 1 \times 10^4$/ml	146	64.3 ± 46.8	6.0 ± 6.3
C	$>1 \times 10^4 \leq 1 \times 10^5$/ml	135	42.5 ± 37.5	6.4 ± 4.3
D	$>1 \times 10^5 < 10 \times 10^6$/ml	688	43.8 ± 24.6	7.7 ± 7.8

[a] Mean ± SD.

Table II. The Outcome of Intracytoplasmic Sperm Injection in the Different Study Groups

Group	Sperm concentration	Injected oocytes	Injected per case	2 PN		1 PN		3 PN	
				No.	%	No.	%	No.	%
A	Cryptozoospermia	1116	10.4 ± 5.2	506	46[a–d]	72	6	30	3
B	≤1 × 10^4/ml	1188	9.7 ± 6.6	625	52[a,c]	65	5	58	5
C	1 × 10^4 ≤ 1 × 10^5/ml	1377	10.1 ± 6.8	748	54[b]	67	5	65	5
D	>1 × 10^5 < 10 × 10^6/ml	6466	9.4 ± 6.4	3970	61[d]	357	5	311	5

[a] $P < 0.0001$.
[b] $P < 0.0001$.
[c] $P < 0.001$.
[d] $P < 0.0001$.

Table III. Cleavage Rate and Embryo Morphology According to Sperm Concentration %

Group	Sperm concentration	Cleavage rate x		Excellent and good embryos		24-hr 4-cell embryos		No. replaced embryos	No. embryos per transfer	Frozen embryos	
		No.	%	No.	%	No.	%			No.	%
A	Cryptozoospermia	437	86	190	67[a,b]	113	40[c,d]	284	2.8 ± 1.4	42	10[a,e,f]
B	≤1 × 10^4/ml	577	92	254	75	176	52	340	2.9 ± 1.1	111	19[e]
C	1 × 10^4 ≤ 1 × 10^5/ml	691	92	284	77[b]	196	53[d]	369	2.9 ± 1.0	108	16[f]
D	>1 × 10^5 < 10 × 10^6/ml	3676	93	1581	78	1089	51[c]	2038	3.1 ± 1.3	633	17[a]

[a] $P < 0.01$.
[b] $P < 0.03$.
[c] $P < 0.001$.
[d] $P < 0.05$.
[e] $P < 0.0001$.
[f] $P < 0.01$.

prerequisite for successful ICSI is the presence of one spermatozoon per oocyte (1,3,11). The relevance of extreme oligozoospermia on the outcome of ICSI is as yet uncertain (3,12). Nagy et al. (3) noted lower fertilization rates in cases with initially low sperm concentration, but concluded that the results of ICSI were not related to sperm concentration. Palmero et al. (12) were not consistent in their opinion on the influence of sperm concentration on fertilization performing ICSI procedure. However, both studies did not refer to such extreme oligozoospermia as appears in the current study. The most severe cases in the study of Nagy et al. (3) were those in which no sperm were found in the grids of the Makler chamber. This group correlates to our group B. In the study of Palmero et al. (12) the lowest sperm concentration is even higher than the one mentioned by Nagy et al. (3).

In this study, we have demonstrated that patients with extreme low sperm count (cryptozoospermia) achieved significantly less fertilizations compared with patients having sperm concentration of ≥10,000 / ml or more (groups B, C, D). There could be several

Table IV. Implantation, Clinical Pregnancies and Abortions in the Different Groups According to Sperm Concentration

Group	Sperm concentration	Implantation		Clinical pregnancies		Abortions	
		No.	%	No.	%	No.	%
A	Cryptozoospermia	28/284	10	20/98	20[a]	6/20	30
B	≤1 × 10^4/ml	46/340	13	35/114	31	6/35	17
C	>1 × 10^4 ≤1 × 10^5/ml	46/369	12	37/133	27	8/37	22
D	>1 × 10^5 <10 × 10^6/ml	267/2038	13	200/640	31[a]	30/200	15[b]

[a] $P < 0.05$.
[b] $P < 0.03$.

explanations for these results. First, in cases with sporadic available spermatozoa it is not feasible to make any morphological analysis. In spite of choosing the sperm for injection with acceptable morphological appearance still some pathologies that could be visible under staining conditions are not recognizable. Sperm with abnormal morphology are known to cause lower fertilization, cleavage, and pregnancy rates (2,5). Another explanation could be a high incidence of DNA fragmentation in poor-quality sperm which has a negative correlation with fertilization rate (13). Likewise, sperm from men with questionable fertility show disturbances in centrosome function and aster formation that are essential for successful fertilization (14). A different explanation is based on the indication that there are lower fertilization rates in couples whose male partner has a chromosomal aberration in comparison with unaffected males (15).

It is well documented that with extreme low sperm concentrations the incidence of chromosome abnormalities is higher (16–18). Chandley et al. (19) found a frequency of 16% of sex chromosomal abnormalities in males with severe oligozoospermia. Kjessler et al. (20) showed that a total of 21.6% of azoospermic men and 3.9% of oligozoospermic males with a sperm count of approximately 1×10^6/ml had an abnormal karyotype. Actually, not only is fertilization rate influenced by sperm quality but sperm quality was shown also to influence embryonic and blastocyst development (21–23) implantation and pregnancy rates and even spontaneous abortions (24–26), as the longest cleavage stage is linked to embryonic genomic activation (22,27).

Indeed, in this study we found a significant effect of sperm concentration on embryo quality and developmental rate. The cases with cryptozoospermia had a significantly lower percentage of embryos with good morphology and significantly fewer four-cell embryos 40–44 hr after egg retrieval compared with cases where sperm concentration was $\leq$10,000 sperm/ml (group B), $\geq$ 10,000 $\leq$ 100,000 (group C), or $>$100,000 and $<$ 10 million/ml (group D). The spouses of patients with cryptozoospermia achieved significantly fewer clinical pregnancies (20%) compared with the pregnancies achieved by the women whose male partners had a sperm concentration of $>$100,000 and $< 10 \times 10^6$/ml (31%). Also, the number of available embryos for freezing was significantly lower in the group of cryptozoospermia compared with all other groups. However, blastocyst development was not examined.

The percentage of miscarriages was significantly higher in the cryptozoospermic population than in the control group. This observation coincides with that of Nagy et al. (3), who found a higher spontaneous abortion rate in women undergoing ICSI using sperm from men with cryptozoospermia.

In conclusion, the outcome of ICSI in severely oligozoospermic–cryptozoospermic patients is poorer than in patients with relative oligozoospermia or control populations in terms of fertilization rate per injected oocyte, embryo quality, embryo developmental rate, cohort of frozen embryos, and of course as a result a lower ongoing pregnancy rate per replaced embryo. Since sperm quality might be affected by causes that are related to severe oligoazoospermia and at the same time is known to be involved in fertilization as well as in further embryo developmental processes, we can speculate that genetic etiology or damaged sperm DNA are responsible for those results.

Thus, a routine of karyotyping patients with sperm counts under 2×10^6 cells/ml should be established. Although patients with inherited de novo Y chromosome deletions cannot be found by this screening method and results of ICSI are poorer in patients with cryptozoospermia, this mode of treatment still achieves normal pregnancies and deliveries, and therefore should be offered to such patients. More than that it is logical to continue the use of ejaculated sperm before testicular sperm extraction is performed as a method for obtaining sperm.

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