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Using ejaculated, fresh, and frozen-thawed epididymal and testicular spermatozoa gives rise to comparable results after intracytoplasmic sperm injection*

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Objective: To describe the preparation of fresh or frozen-thawed epididymal and testicular sperm for intracytoplasmic single sperm injection and to compare the fertilization, embryo quality, and pregnancy rates (PRs) obtained after using these spermatozoa to the results when freshly ejaculated sperm was used for microinjection.

Design: Retrospective analysis of 1,034 consecutive microinjection cycles. Ejaculated (965 cycles), fresh epididymal (43 cycles), frozen-thawed epididymal (9 cycles), and testicular sperm (17 cycles) was used for intracytoplasmic sperm injection.

Setting: Procedures were performed in a tertiary IVF center coupled with an institutional research environment.

Main Outcome Measures: Semen density and motility were judged by the World Health Organization criteria and sperm morphology was evaluated by the Tygerberg's strict criteria. After microinjection, oocyte intactness, fertilization, embryo cleavage, transfer, and PRs were evaluated and compared.

Results: The median values of total sperm count, total motility and normal morphology were 17.85×10^6, 37%, 8% for freshly ejaculated sperm; 46.20×10^6, 12%, 9% for fresh epididymal sperm; 0.15×10^6, 0%, 0% for frozen-thawed epididymal sperm; and 0.54×10^6, 0% for testicular sperm (morphology was not determined). The percentage of intact oocytes after microinjection ranged from 84% to 90%. Normal fertilization rates were high when fresh or frozen-thawed epididymal and testicular spermatozoa were used for the injection (56%, 56%, 48%, respectively) but were significantly lower than for ejaculated sperm (70%). There was a higher proportion of transferable embryos obtained after ejaculated sperm injection than after testicular sperm injection. Forty percent, 58%, 33%, and 46% of cycles had positive serum hCG using ejaculated, fresh, or frozen-thawed epididymal and testicular sperm. Initial pregnancy loss occurred in 26.3% of the conception cycles.

Conclusion: Intracytoplasmic sperm injection can provide high normal fertilization, cleavage, and PRs when fresh or frozen-thawed epididymal and testicular spermatozoa are used, but normal fertilization rates are significantly lower than after microinjection with ejaculated sperm.

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Key Words: Intracytoplasmic sperm injection, epididymal sperm, male infertility, microsurgical epididymal sperm aspiration, microinjection, testicular sperm

The alleviation of male factor infertility by standard IVF procedure has been applied widely, but

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the outcome frequently has been disappointing as a result of the usually low fertilization rate (1). To improve fertilization, modifications have been introduced in the standard IVF procedure, such as heavy insemination (2), and several new procedures of assisted fertilization have been introduced, such as partial zona dissection (3) and subzonal insemination (4, 5). None of these procedures has helped to increase substantially the fertilization and pregnancy rates (PRs). A few years after its first introduction (6), much higher fertilization and PRs were obtained via intracytoplasmic sperm injection (7–9).

This fact and the unique condition that intracytoplasmic injection requires only a single spermatozoon per oocyte have led to the use of the intracytoplasmic sperm injection technique where fresh or frozen-thawed epididymal and testicular sperm were to be used for insemination (10, 11). The aim of this study was to analyze retrospectively the oocyte survival, fertilization, cleavage, and PRs when fresh or frozen-thawed epididymal and testicular sperm was used for intracytoplasmic injection and to compare these data to the results of intracytoplasmic sperm injection where ejaculated sperm was used. In the present study, 1,034 treatment cycles were analyzed, which were performed between October 1992 and December 1993.

MATERIALS AND METHODS

Patients

The patients' inclusion criteria for intracytoplasmic sperm injection with freshly ejaculated semen were either failed or very low (≤10%) fertilization rate in the previous standard IVF cycle(s) or extremely poor sperm parameters (e.g., <500,000 motile spermatozoa in the whole ejaculate) precluding the inclusion of the patient in a standard IVF procedure. Microsurgical epididymal sperm aspiration was performed on patients who had failed reversal of vasectomy or vasoepididymostomy or who were infertile as a result of congenital bilateral absence of the vas deferens. The medical workup of these patients included semen analysis, transrectal ultrasonography, endocrinologic profile and for patients with congenital bilateral absence of the vas deferens, screening for the commonest cystic fibrosis mutations (10). Testicular tissue biopsies were performed when either no sperm was found in the epididymis or the epididymis was missing as a result of previous surgical interventions.

Ovarian Stimulation

Ovarian stimulation was carried out by a desensitizing protocol using the GnRH agonist buserelin acetate (Suprefact; Hoechst, Brussels, Belgium) in combination with hMG (Humegon; Organon, Oss, The Netherlands; or Pergonal; Serono, Brussels, Belgium) and hCG (Pregnyl; Organon; or Profasi, Serono). For luteal phase supplementation, intravaginally administered P (Utrogestan; Piette, Brussels, Belgium) was used. The details of this stimulation protocol have been described previously (12).

Semen Evaluation and Preparation

Sperm density and motility were evaluated according to the recommendations of the World Health Organization (13). Morphology was evaluated by Tygerberg's strict criteria (14). A semen sample was considered to be normal when sperm density was $>20 \times 10^6$ /mL, progressive motility was >40%, and ≥14% of the spermatozoa had normal morphology.

The sperm treatment procedure of the freshly ejaculated semen has been described previously (15). Semen was washed in Earle's medium by centrifuging for 5 minutes at 1,800 × *g* after a 30-minute liquefaction period. The pellet was put on a two- or three-layer Percoll gradient (95% to 47.5% or 90% to 70% to 50%) and then was centrifuged at 300 × *g* for 20 minutes. The 95% or 90% Percoll fraction was washed again with Earle's medium for 5 minutes at 1,800 × *g* and the pellet was recentrifuged in Earle's medium just before microinjection.

Epididymal semen usually was recovered from the most proximal part of the caput of the epididymis. During microsurgical epididymal sperm aspiration, several sperm fractions were collected into separate tubes. Sperm fractions with the highest concentration and motility were pooled and then treated in the same way as ejaculated semen. Whenever possible, a part of the freshly recovered epididymal semen was frozen (16) for later use to avoid the microsurgical epididymal sperm aspiration procedure in subsequent cycles.

Immediately after thawing, frozen-thawed epididymal semen was put on a two-layer Percoll (95% to 47.5%) and was centrifuged for 20 minutes at 300 × *g*. The 95% fraction was washed with Earle's medium at 1,800 × *g* for 5 minutes and the pellet was used for injection after one additional centrifugation in a 1.5-mL Eppendorf tube (1,800 × *g*, 5 minutes).

Testicular sperm was prepared by dicing and squeezing a piece of testicular tissue in HEPES-buffered Earle's medium and by centrifuging this medium for 5 minutes at 300 × *g*. The pellet was kept without any further treatment until the moment of injection.

Oocyte Preparation

Cumulus-corona-oocyte complexes were retrieved by vaginal ultrasound-guided puncture performed 36 hours after hCG administration. The cells of the cumulus and corona radiata were removed by incubation of the cumulus-corona-oocyte complexes for <1 minute in HEPES-buffered Earle's medium, containing 80 IU/mL hyaluronidase (type VIII, specific activity 320 IU/mg; Sigma Chemical Co, St. Louis, MO) and by aspiration of the cumulus complexes in and out of a hand-drawn glass pipette (diameter approximately 200 μm). The denuded oocytes were rinsed several times, first in HEPES-buffered Earle's medium, and then in B2 medium (bioMérieux, Montalieu Vercieu, France). Nuclear maturity of the oocytes was judged under an inverted microscope at a ×200 magnification. Until the moment of injection, the oocytes were kept in 25-μL microdrops of B2 medium covered by lightweight paraffin oil (British Drughouse; Pasture, Brussels, Belgium) in an incubator at 37°C in an atmosphere of 5% CO_2; 5% O_2, and 90% N_2.

Intracytoplasmic Sperm Injection Procedure

The details of microtool preparation and microinjection procedures have been described previously (8, 9). The essential steps in the intracytoplasmic sperm injection procedure can be summarized as follows: Holding and injection pipettes were made from 30-μL borosilicate glass capillary tubes (Drummond Scientific Company, Broomall, PA). After extensive washing they first were pulled on a horizontal microelectrode puller (type 753; Campden Instruments Ltd, Loughborough, United Kingdom) and then, with the help of a microginder and microforge, a sharp opening was prepared at the end of the injection pipette. The injection pipette had a 5 to 6 μm inner diameter and a 7 to 8 μm outer diameter while the holding pipette had a 10 to 20 μm inner diameter and a 60 to 80 μm outer diameter.

The injection dish contained eight droplets of 5 μL HEPES-buffered Earle's medium. The central droplet contained 10% polyvinylpyrrolidone (PVP, P5288; Sigma) and 1 μL of resuspended sperm pellet was added if the sperm concentration was high enough after the selection procedure. If the initial sperm parameters were extremely poor, 5 μL of pelleted sperm was put separately into the injection dish at the end of the sperm treatment, next to the droplet of medium containing 10% PVP.

The intracytoplasmic sperm injection procedure was carried out on the heated stage of an inverted microscope (Diaphot; Nikon Corporation, Tokyo, Japan) at ×400 magnification using the Hoffman Modulation Contrast System (Modulation Optics Inc., Greenvale, New York). The holding and injection pipettes were placed into a tool holder and were connected to a micrometer-type microinjector (IM-6; Narishige, Tokyo, Japan). The movements of the pipettes were coordinated by two coarse positioning manipulators (3D Motor Driven Coarse Control Manipulator MM-188; Narishige) and with two three-dimensional hydraulic remote-control micromanipulators (Joystick Hydraulic Micromanipulator MO-188; Narishige).

A single living immobilized spermatozoon was aspirated tail first into the injection pipette directly from the PVP-sperm droplet or taken from the separate sperm droplet and than transferred to the PVP droplet to clean the sperm first from the attaching cells and debris. For the injection, the oocyte was fixed on the holding pipette in a way that the polar body was situated at 12 o'clock or at 6 o'clock while the injection pipette was pushed through the zona pellucida at the 3 o'clock position and into the cytoplasm, where the sperm was delivered together with the smallest possible amount of medium (approximately 1 pL of medium). After injection the oocytes were washed and stored in 25-μL microdrops of B2 medium in a petri dish and stored in an incubator containing 5% CO_2, 5% O_2, and 90% N_2.

Assessment of Fertilization and Embryo Cleavage and Establishment of Pregnancy

Sixteen to 18 hours after microinjection, oocytes were inspected for survival and fertilization (17). The number and aspect of polar bodies and pronuclei were recorded. The criteria for normal fertilization were the presence of two individualized or fragmented polar bodies together with two clearly visible pronuclei. Embryo cleavage and quality were evaluated 40 to 44 hours after intracytoplasmic sperm injection. According to the relative proportion of anucleate fragments present in the zona pellucida, they were assigned to one of the four catego-

ries: [1] excellent, no anucleate fragment was present; [2] good, <20% of the embryo was fragmented; [3] fair, the relative amount of fragments was between 20% and 50%; and [4] poor, >50% of the embryo was fragmented. Embryos with <50% fragmentation were eligible for transfer. Supernumerary embryos with <20% fragmentation were cryopreserved (18). The further cleavage of supernumerary embryos of fair quality was observed of a period of 5 days. They were cryopreserved if they reached the blastocyst stage. To avoid multiple pregnancies, especially triplet pregnancies, only two embryos were transferred in some selected cases. The patient selection criteria for transfer of only two embryos were first or second treatment cycle, <37 years of age, and large number of excellent or good-quality embryos (19). Exceptionally, in some older patients, four embryos were replaced.

Pregnancy was confirmed when serum hCG concentrations were rising on at least two separate occasions 10 days after embryo transfer. Clinical pregnancy was determined by detecting a gestational sac by ultrasonography at approximately 7 weeks of pregnancy.

Patients were counseled and agreed to have prenatal diagnosis by chorionic villus sampling or by amniocentesis. Patients also agreed to participate in a follow-up study of the children born after microinjection.

Statistics

Statistical tests were performed at the 5% level of significance. The calculations were carried out using the SPSS statistical package (SPSS Inc., Chicago, IL) on an Inwork (Inelco, Brussels, Belgium) personal computer.

Intactness, fertilization, embryo quality, embryo transfer, and embryo freezing rates were compared globally by the Kruskal-Wallis test. If significant differences occurred between groups, then paired comparisons were performed by means of the Mann-Whitney *U*-test. These tests were performed on the percentage values of the variables within each cycle. This approach was used to take the dependence of observations within each cycle into account and it is necessary for a valid application of the tests of comparisons, which assume independence of the observations. Pregnancy rates were compared by means of the χ^2 test.

RESULTS

The semen characteristics in the 965 treatment cycles with freshly ejaculated semen are summarized in Table 1. In 6% of cycles, all semen parameters were normal, in 16% of the cycles there was a single sperm defect present, in 28% of the cycles there was a double sperm defect, whereas in 50% of the cycles a triple sperm anomaly was detected. The standard semen parameters of freshly recovered epididymal, frozen-thawed epididymal, and testicular sperm are summarized in Table 2. The total number of spermatozoa varied greatly from patient to patient as indicated by the wide range. The total motility and progressive motility of the freshly recovered epididymal sperm was usually very low and decreased further after freezing-thawing. There also was wide variation from sample to sample. The percentage of spermatozoa with normal morphology was reduced, especially in frozen-thawed epididymal samples. Testicular sperm always had only a slow sluggish local motility. The number of motile spermatozoa was especially low in the first minutes after recovery from the biopsy specimen and increased after 1 to 2 hours of in vitro incubation. Morphology classification was not carried out on testicular sperm because not enough spermatozoa were available for a correct assessment.

Table 1 Sperm Anomalies in the Freshly Ejaculated Semen Group (n = 965)*

No. of Anomalies	No. of cycles
Normal	61 (6.3)
Single anomaly	154 (16.0)
Oligozoospermia	43 (4.5)
Asthenozoospermia	55 (5.7)
Teratozoospermia	56 (5.8)
Two anomalies	271 (28.0)
Oligoasthenozoospermia	82 (8.5)
Oligoteratozoospermia	125 (13.0)
Asthenoteratozoospermia	64 (6.6)
Three anomalies	
Oligoasthenoteratozoospermia	479 (50.0)

* Values in parentheses are percentages.

In these 1,034 cycles 13,330 cumulus-corona-oocyte complexes were recovered, i.e., a mean of 12.9 cumuli per cycle. The results of intracytoplasmic sperm injection with the different types of semen are summarized in Table 3. Intracytoplasmic sperm injection was carried out on 10,853 metaphase II oocytes that had extruded the first polar body, i.e., 81.4% of the cumulus-corona-complexes contained a fertilizable oocyte. The percentage of oocytes recorded damaged 16 to 20 hours after intracytoplasmic sperm injection ranged from 10% to 15% in the four different semen groups.

The pronuclear status of these intact oocytes revealed that the rate of two pronuclear oocytes was

Table 2 Sperm Parameters in the Four Groups*

Sperm groups	Total count ($\times 10^6$)	Motility†	Progressive motility†	Normal morphology†
		%	%	%
Ejaculated	17.85 (0 to 1,022†)	37 (0 to 100)	22 (0 to 100)	8 (0 to 62)
Fresh epididymal	46.20 (0 to 248)	12 (0 to 49)	2 (0 to 20)	9 (0 to 33)
Frozen-thawed epididymal	0.15 (0 to 4.4)	0 (0 to 5)	0 (0 to 5)	0 (0 to 15)
Testicular	0.54 (0.27 to 3.4)	0 (0 to 15)	0	ND‡

* Values are medians with ranges in parentheses.
† The high total sperm count of $1,022 \times 10^6$ occurred in acouple with four previous IVF failures.
‡ ND, not determined.

significantly higher when microinjection was carried out with ejaculated sperm than with epididymal or testicular sperm. A single pronucleus was present in 3.9%, 6.0%, 1.0%, and 4.6% as a mean of the cycles in the four sperm groups, respectively (statistically not different). The proportion of oocytes with three pronuclei was different ($P < 0.05$) for the four groups of sperm and ranged from 3.9% to 9.0%.

Almost all two-pronuclear oocytes had at least one mitotic division. The morphological quality of the cleaved embryos was similar among the four semen groups. A slightly different distribution in embryo quality was noticed for the good- and poor-quality embryos. Microinjection with ejaculated sperm seems to generate the best-quality embryos. Seventy-six percent of two pronuclear oocytes (as a mean of the cycles) cleaved to transferable embryos after microinjection with ejaculated sperm. The proportion of transferable embryos was slightly lower for freshly collected (73%) and frozen-thawed epididymal sperm (73%) and for testicular spermatozoa (62%). However, these proportions were statistically not different between the four groups only when the testicular sperm group was compared with the ejaculated sperm group alone by the one-way analysis of variance test ($P < 0.05$).

The percentage of embryo replacements was very high in the four sperm groups. However, embryos were replaced in only 13 of 17 cycles (77%) of intracytoplasmic sperm injection with testicular spermatozoa, which was a significantly lower value compared with the other groups. The percentages of two pronuclear oocytes developing to embryos that were actually transferred or frozen was 70% for ejaculated sperm, 68% for freshly collected epididymal sperm, 75% for frozen-thawed epididymal sperm, and 64% for testicular sperm. Higher proportion of embryos were frozen after intracytoplasmic sperm injection with ejaculated sperm (22% of two pronuclear oocytes) than in the three other sperm categories (11%, 10%, and 15%). The replacement of at least one embryo was possible in 960 of 1,034 started cycles (93%). The distribution of number of embryos transferred was different among the four categories of semen. Very few cycles had only one embryo for transfer; in most cycles two or three good-quality embryos were replaced, exceptionally, four embryos were replaced in some of the older patients.

The total number of pregnancies was 393, i.e., 40.9% per transfer or 38.0% per started cycle. The clinical PR per transfer and per started cycle was 30.1% and 28.0%. Further pregnancy loss (ectopic pregnancy or clinical abortion) occurred in 44 pregnancies. The clinical ongoing or delivery rate per transfer and per started cycle was 25.5% and 23.7%.

DISCUSSION

It is clear from our published results that very high fertilization and embryo cleavage rates can be obtained via intracytoplasmic sperm injection using ejaculated semen (8, 9). Intracytoplasmic sperm injection, in contrast to standard insemination, provides high fertilization and embryo developmental rates using epididymal and testicular sperm, but until now there were no data available to compare intracytoplasmic sperm injection results using these different types of spermatozoa.

The distribution of 0 to 3 anomalies among the 965 freshly ejaculated semen samples was similar to the one(s) reported earlier, showing only a very slight shift towards to the group with triple semen anomaly (7, 9). Total sperm count, motility, and morphology values for the fresh and frozen-thawed and testicular spermatozoa are difficult to evaluate because established parameters or ranges do not exist for semen taken from the epididymis or testis. Moreover, total sperm count, motility, and perhaps morphology parameters might be influenced by the

Table 3 Results of Intracytoplasmic Sperm Injection Using Ejaculated, Fresh, and Frozen-thawed Epididymal and Testicular Sperm

		Epididymal		
	Ejaculated	Fresh	Frozen-thawed	Testicular
No. of cycles	965	43	9	17
Mean age of patients (y)				
Female	32.3	31.7	30.6	34.6
Male	35.5	38.7	33.2	42.8
No. of injected oocytes	9,958	547	106	242
Intact oocytes* (%)	89 ± 14	90 ± 13	84 ± 18	90 ± 10
Pronuclear status				
Intact oocytes* (%)				
Two pronuclei†	70 ± 23	56 ± 28	56 ± 25	48 ± 26
Zero pronuclei†	21 ± 22	34 ± 29	34 ± 26	40 ± 31
One pronucleus	4 ± 8	6 ± 10	1 ± 3	5 ± 6
More than two pronuclei‡	5 ± 11	4 ± 8	9 ± 10	7 ± 8
Embryo development of the two pronucleus oocytes				
Cleaved embryos* (%)				
Excellent	9 ± 17	7 ± 17	4 ± 6	4 ± 9
Good	53 ± 29	51 ± 27	37 ± 37	51 ± 33
Fair	14 ± 22	13 ± 19	32 ± 42	7 ± 12
Poor	23 ± 25	27 ± 28	27 ± 23	37 ± 33
No. of embryo transfers§‖	898 (93)	40 (93)	9 (100)	13 (77)
One embryo	64 (7)	2 (5)	1 (11)	1 (8)
Two embryos	324 (36)	8 (20)	3 (33)	1 (8)
Three embryos	486 (54)	24 (60)	4 (44)	7 (54)
More than three embryos	24 (3)	6 (15)	1 (11)	4 (31)
Embroys frozen as percent of the two-pronuclear oocytes*‡	22 ± 26	11 ± 23	10 ± 20	15 ± 22
No. of embryos transferred	2.4 ± 0.9	2.7 ± 1.0	2.6 ± 0.9	2.5 ± 1.8
No. of pregnancies¶				
Positive hCG	361 (40)	23 (58)	3 (33)	6 (46)
Clinical pregnancies	269 (30)	12 (30)	3 (33)	5 (39)
Unknown	14 (2)	2 (5)	—	—

* Values are expressed as a mean ± SD.

† Significant difference ($P < 0.001$) between groups by the Kruskal-Wallis test.

‡ Significant difference ($P < 0.05$) between groups by the Kruskal-Wallis test.

§ Significant difference ($P < 0.05$) between groups by the χ^2 test (the frozen-thawed epididymal sperm group is disregarded because of too small expected frequencies).

‖ Values in parentheses are percentages.

¶ Values in parentheses are percentages (pregnancy per transfer).

place and size of the incision on the epididymis and testis made during the microsurgical intervention (20). Nevertheless, it was possible to recover enough motile spermatozoa for microinjection on all occasions. Although the total number of spermatozoa obtained from the epididymis was usually high, only slow progressive or just local motility was typically observed. In a recent publication the reported numbers and motility of fresh epididymal sperm were in the same range as we found (21). The total free sperm count of testicular sperm was always low and in a narrow range, but never zero. An unexpected feature of the testicular sperm was that the zero motility rate, which was usually observed just a few minutes after the biopsy, rose with time and 0.5 to 2 hours later several sperm cells showed local motility. There is no explanation at the moment for this observation but it is conceivable that certain motility blocking factors, which are present in the testes, are diluted out during sperm treatment, leading to an increase of the motility of some testicular sperm cells later on. It was not possible to conduct an accurate and informative morphological examination of the testicular spermatozoa because of the very few free sperm cells present in the smear, but the few that were free resembled ejaculated sperm with normal morphology, except that many cells had cytoplasmic droplets.

The injection procedure usually required more time per oocyte when frozen-thawed epididymal or testicular sperm was used than was the case with ejaculated sperm. The reason for this was that one or sometimes even three to four extra sperm droplets needed to be set in the injection dish from where a single sperm was to be aspirated (carefully avoiding the aspiration of the debris that can block

the pipette) and transferred into the central droplet containing 10% PVP, where it was washed before injecting into an oocyte. The inclusion of extra sperm droplets was necessary for two main reasons. One reason was the very low number of spermatozoa present in the pellet after the final centrifugation (concentration step) in cases of frozen-thawed epididymal and testicular sperm; the other reason was that the pellet contained a lot of debris at the end of the sperm selection procedure, making it necessary to clean the sperm from attached cells or debris. Testicular and frozen-thawed epididymal sperm especially required extensive cleaning-washing procedures in the PVP droplet. To ease this cumbersome injection procedure for testicular spermatozoa, it is worth trying the Percoll gradient centrifugation as a sperm cleaning-selection procedure in those cases where relatively high numbers of sperm cells are present in the testicular biopsy.

Although the injection procedure for epididymal and testicular sperm was more awkward than for ejaculated sperm, it did not result in a higher oocyte degeneration rate. The fertilization rates observed after fresh and frozen-thawed or testicular sperm injection were unexpectedly high (48% to 56%) but still significantly lower than after ejaculated sperm injection (70%). This difference in the fertilization rate might be related to the incomplete maturation process of epididymal and testicular sperm. Strikingly, no difference was observed in the fertilizing capacity of the testicular and epididymal sperm. This suggests that there is no specific process to enhance the pronucleus formation potential of the sperm in the epididymis, at least not in those men who require microsurgical epididymal sperm aspiration.

A similar parthenogenetic activation rate was observed in all the groups (4% to 6%), except in the frozen-thawed epididymal sperm group, where the rate was only 1%. But because of the few patients in this group (nine patients), it would be premature to draw conclusions from this. The rate of three-pronuclear zygotes was different among the four sperm groups (highest in the frozen-thawed epididymal sperm group). In many cases these three-pronuclear oocytes exhibited only one polar body. The occurrence of three-pronuclear oocytes after single sperm injection is puzzling and needs to be studied further (17).

A slight difference was present in embryo quality when testicular sperm was used as compared with ejaculated sperm. It is difficult to interpret this difference because it is not clear how a sperm originating from different sources can influence embryonic development after normal fertilization has taken place. There are two recent communications that report observations of similar events, in which a connection has been found between embryo quality and sperm parameters. Perinaud et al. (22) observed lower embryonic quality in relation to sperm morphology in normal IVF and Cohen et al. (23) made similar observations after partial zona dissection. Because morphologically abnormal sperm does not appear to carry more abnormal chromosomes (24), further investigations are needed to identify possible biochemical or genetic factors behind these observations.

As a result of the high fertilization and embryo cleavage rates, the majority of the patients in each group had embryo replacements, allowing the transfer of two or more embryos for most of the patients. Only four patients did not have embryo replacement in the testicular sperm group: two of them had no fertilization, the other two showed poor cleavage.

The rate of positive serum hCG (total PR) was high in each patient population. The very high total PR in the fresh epididymal and, especially, in the testicular sperm groups was partly the result of multiple embryo transfer. The overall pregnancy loss of 37.7% is in the range usually obtained among patients undergoing standard IVF-ET procedures. So far, the results of prenatal karyotypes and the prospective pediatric follow up of the children born after microinjection do not indicate an increase in congenital malformations (25).

It is obvious from this study that it is possible to achieve high normal fertilization, embryo cleavage, and PRs via intracytoplasmic sperm injection with appropriately treated epididymal and testicular sperm, although the fertilization rates are lower than the fertilization rate using freshly ejaculated sperm. This lower fertilization rate obtained after the use of epididymal or testicular sperm is a possible consequence of the type of sperm used.

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