Frontiers in Endocrinology
Volume 11 - 1995

Embryo Development after *ICSI* Using Testicular, Epididymal and Ejaculated Spermatozoa

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INTRODUCTION

In recent years intracytoplasmic sperm injection (ICSI) has been applied successfully in couples with andrological infertility who could not be helped by standard IVF treatment or who were not accepted for IVF because the number of motile spermatozoa in the ejaculate was too low (5), (6), (13), (11), (12). ICSI was also used in patients requiring microsurgical sperm aspiration because of obstructive azoospermia. ICSI with epididymal spermatozoa yielded better results than conventional IVF (7), (10). It was also possible to obtain normal fertilization and pregnancies as a result of ICSI only with spermatozoa retrieved from a testicular biopsy specimen (2), (4), (15).

In this report a retrospective analysis has been carried out of 1034 consecutive ICSI cycles performed during a 14-month period (October 1992 until December 1993). Oocyte survival, fertilization, cleavage and pregnancy rates were compared when ICSI was carried out with ejaculated spermatozoa, fresh or frozen-thawed epididymal spermatozoa or testicular spermatozoa.

through a Percoll gradient and iii) a concentration step just prior to microinjection.

Fresh epididymal sperm was recovered by MESA; the sperm fractions with the highest concentration and motility were pooled and processed like ejaculated sperm; wherever possible freshly recovered epididymal sperm was frozen in order to avoid microsurgery in subsequent cycles (16). Frozen-thawed epididymal sperm was put on a two-layer Percoll gradient, and then processed like ejaculated sperm. Testicular sperm was prepared by dicing and squeezing a piece of testicular tissue in buffered culture medium and by centrifuging this medium for 5 min. at 300 g. There was a wide variation in the number of spermatozoa present in epididymal fluid or testicular tissue. Motility was reduced for both types of spermatozoa. In most treatment cycles it was possible to find enough spermatozoa with some motility in the prepared semen to inject all MII oocytes.

The details of the microinjection procedure have been described in detail previously (11), (12). Essential steps in the ICSI procedure can be summarized as i) the preparation of holding and injection pipettes, ii) the preparation of the Petri dish with the central sperm droplet containing 10% polyvinylpyrrolidone (PVP) and iii) the injection procedure itself where a single spermatozoon was inserted into each of the metaphase II oocytes. The injected oocytes were washed carefully and stored in 25 µl of B2 medium covered by paraffin oil in an incubator containing 5% CO_2, 5% O_2 and 90% N_2.

DAMAGE AND FERTILIZATION AFTER ICSI

Oocytes were inspected for intactness and for fertilization 16 to 18 h after the ICSI procedure (3). Oocytes were considered to be normally fertilized if they had two individualized or fragmented polar bodies and two distinct pronuclei with nucleoli. As indicated in Table 1, the proportions of intact oocytes were similar in the four groups of sperm and ranged from 84% to 90% of the injected oocytes. The proportion of two-pronuclear oocytes was significantly higher (70%) where ICSI was carried out with ejaculated sperm than with either epididymal or testicular sperm (between 48% and 56%). The mean percentages of oocytes with one pronucleus were similar for the four different semen categories. The proportions of oocytes with 3-PN were different for the four groups of sperm.

PATIENT MANAGEMENT

ICSI was carried out with ejaculated spermatozoa in 965 cycles where either previous IVF cycles had failed or the sperm parameters precluded standard IVF treatment.

ICSI was performed with fresh epididymal spermatozoa in 43 cycles and with frozen-thawed epididymal spermatozoa in 9 cycles. Epididymal spermatozoa were collected by microsurgical sperm aspiration (MESA) in patients with obstructive azoospermia due to congenital bilateral absence of the vas deferens (CBAVD) or failed reversal of vasectomy or vaso-epididymostomy.

ICSI was carried out in 17 cycles with spermatozoa retrieved from a testicular biopsy specimen because either there were no motile spermatozoa present in the epididymides or the epididymides were missing after previous surgery.

INTRACYTOPLASMIC SPERM INJECTION PROCEDURE

Oocytes were obtained after ovarian stimulation with gonadotrophin-releasing-hormone agonist, human menopausal gonadotrophins (HMG) and human chorionic gonadotrophins (HCG); the luteal phase was supplemented with intravaginally administered progesterone (8).

Oocyte retrieval was carried out 36 h after HCG administration. In all, 13 330 cumulus-corona-oocyte complexes were retrieved i.e. a mean of 12.9 cumuli/cycle. The cells of the cumulus oophorus and the corona radiata were removed by a combination of an enzymatic and a mechanical procedure. The nuclear maturity of the oocytes was then assessed under an inverted microscope at x200 magnification. The first polar body had been extruded in 10 583 oocytes, i.e. 81.4% of the cumulus-oocyte complexes contained metaphase II oocytes capable of fertilization.

Sperm density and motility were evaluated according to WHO criteria and sperm morphology by strict Tygerberg criteria. The semen characteristics of the 965 cycles with ejaculated sperm revealed i) a triple sperm defect in 50% of the cycles, ii) a double sperm defect in 28% of the cycles, iii) a single sperm defect in 16% of the cycles and iv) normal semen parameters in 6% of the cycles. Ejaculated semen was prepared for ICSI by i) washing out the seminal fluid, ii) passage

Clinical pregnancy was determined by detecting a gestational sac by ultrasonography.

Most two-pronuclear oocytes had at least one mitotic division. The morphological quality of the cleaved embryos was similar across the four semen groups (Table 2). The proportion of transferable embryos was slightly higher with ejaculated sperm but a statistically significant difference was observed only by one-way ANOVA test between the testicular and the ejaculated sperm group. The percentage of 2-PN oocytes developing to embryos which were actually transferred or frozen ranged from 75% to 64% (Table 2). A higher proportion of embryos were frozen after ICSI with ejaculated sperm (22% of 2-PN oocytes) than in the three other sperm categories (between 10% and 15% of 2-PN oocytes).

The percentage of cycles with embryo replacement was very high in the four groups (between 77% and 100%). Transfer was possible in 13 of 17 cycles with testicular spermatozoa; this transfer rate was significantly lower than in the other groups. The mean number of embryos transferred was similar in the four groups and ranged from 2.4 to 2.7 embryos per transfer.

The total number of pregnancies was 393, i.e. 41% per transfer or 38% per started cycle. The clinical ongoing pregnancy or delivery rates per transfer and per started cycle were 26% and 24%.

Because of the novelty of these procedures patients were counselled about the many unknown aspects and agreed to have prenatal diagnosis and to participate in a prospective follow-up study of the children born after microinjection (1).

TABLE 1. Intactness and fertilization after ICSI using ejaculated, fresh and frozen-thawed epididymal and testicular sperm

	Ejaculated	Epididymal		Testicular
		Fresh	Frozen-thawed	
Cycles	965	43	9	17
Injected oocytes	9958	547	106	242
% of intact oocytes	89	90	84	90
% of intact oocytes with:				
PN[2]	70	56	56	48
1PN	4	6	1	5
≥3PN[2]	5	4	9	7

1 P<0.001 between groups by the Kruskal-Wallis test
2 P<0.05 between groups by the Kruskal-Wallis test

EMBRYO CLEAVAGE

About 24 h after further in-vitro culture (40-44 h after the ICSI procedure), embryo cleavage was evaluated. Cleaved embryos were categorized into four groups according to the relative proportion of anucleate fragments present : 1) excellent, where no anucleate fragments were present, 2) good, where less than 20% of the embryo was fragmented, 3) fair, where the relative proportion of fragments was between 20 and 50% and 4) poor, where more than 50% of the embryo was fragmented. Embryos with less than 50% fragmentation were eligible for transfer. Only two embryos were transferred in some selected cases in order to avoid triplet pregnancies (9). Exceptionally, four embryos were replaced, especially in older patients. Supernumerary embryos with less than 20% fragmentation were cryopreserved (14).

Pregnancy was confirmed where serum HCG concentrations were rising on at least two separate occasions 10 days after embryo transfer.

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TABLE 2 : Embryo cleavage and transfer after ICSI using ejaculated, fresh and frozen-thawed epididymal and testicular sperm

	Ejaculated	Epididymal		Testicular
		Fresh	Frozen-thawed	
Embryo development % of cleaved embryos				
Excellent	9	7	4	4
Fair	53	51	37	51
Poor	14	13	32	7
% of transferable embryos	76	73	73	62
% of transferred or frozen	70	68	75	64
% of cycles with transfer[1]	93	93	100	77
Embryos transferred	2.4	2.7	2.6	2.5
Pregnancy rate per transfer				
Positive HCG	40%	58%	33%	46%
Clinical	30%	30%	33%	39%

(1): $P<0.05$ between groups by the chi-square test (the frozen-thawed epididymal sperm is disregarded because of too small expected frequencies).