Higher pregnancy rates using testicular sperm in men with severe oligospermia

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Objective: To evaluate assisted reproductive technology (ART) outcomes using testicular sperm in oligospermic men who previously failed to achieve paternity using TUNEL-positive ejaculated sperm.
Design: Retrospective cohort.
Setting: Academic medical center.
Patient(s): Twenty-four oligospermic men who failed one or more ART cycles using ejaculated sperm with TUNEL-positive proportion >7%, and subsequently underwent microsurgical testicular sperm extraction (TESE).
Intervention(s): TESE followed by intracytoplasmic sperm injection (ICSI).
Main Outcome Measure(s): TUNEL-positive level in ejaculated and testicular sperm; clinical pregnancy.
Result(s): The mean TUNEL-positive level was 24.5% for ejaculated sperm, and 4.6% for testicular sperm. Clinical pregnancy was achieved in the first ART cycle with testicular sperm in 12 (50%) out of 24 couples. There was no statistically significant difference in maternal and paternal age, maternal gravity and parity, number of previous ART attempts, concentration or motility of retrieved sperm, number of oocytes retrieved, fertilization rate, or number of embryos transferred between couples who did and did not achieve pregnancy. No miscarriages occurred. All 12 pregnancies resulted in the delivery of healthy children.
Conclusion(s): The percentage of TUNEL-positive cells is lower in testicular sperm for oligospermic men who have abnormal ejaculated sperm DNA fragmentation. The use of testicular sperm for ICSI was associated with a 50% pregnancy and live-birth rate for couples who had previously failed one or more IVF–ICSI cycles with ejaculated sperm. No other clinical predictors of successful pregnancies after the use of surgically retrieved sperm could be identified. In men with elevated TUNEL-positive ejaculated sperm and failed ART, TESE may be considered. (Fertil Steril® 2015;104:1382–7. ©2015 by American Society for Reproductive Medicine.)
Key Words: Infertility, recurrent pregnancy loss, sperm DNA fragmentation, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, TESE, testicular sperm extraction

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Excepting men with nonobstructive azoospermia, the vast majority of patients with male factor infertility have sufficient sperm in the ejaculate for use with intracytoplasmic injection (ICSI). Ejaculated spermatozoa that have completed maturation during their transit through the male reproductive tract generally have better fertilization potential than testicular sperm (1). Nevertheless, with advances in sperm preparation and selection methods, as well as advances in the techniques of assisted reproductive technology (ART), the pregnancy rates after the use of testicular versus ejaculated sperm for ICSI have been shown to be comparable among men with similar etiologies of male factor infertility (2).

Fewer studies have directly compared fertility outcomes after the use of ejaculated or testicular sperm within the same cohort of patients. Weissman et al. (3) reported improved embryo implantation rates and pregnancy rates using testicular sperm compared with ejaculated sperm in four couples with severe oligoasthenoteratozoospermia syndrome. Similarly, Hauser et al. (4) and Ben-Ami et al. (5) reported improved implantation and

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pregnancy rates with the use of fresh testicular versus fresh ejaculated sperm in men with either severe oligospermia or cryptozoospermia. Taken together, the results of these studies appear to favor the use of testicular sperm over ejaculated sperm in men with severe male factor infertility.

A growing body of literature suggests that sperm from infertile men contains more DNA damage than sperm from fertile men (6, 7), and that the degree of sperm DNA damage can negatively impact the fertility potential of affected men (8, 9). Even when sperm are selected to avoid those spermatozoa with gross DNA damage, the outcome of assisted reproduction is affected when the neat (unprocessed) semen sample has increased sperm DNA damage. When used for assisted reproduction, sperm from a sample with increased DNA damage may be able fertilize an oocyte (10), but the resulting embryo may fail to develop or implant, or may be naturally aborted at a later stage (11).

Although delays in sperm transport, varicoceles, and gonadotoxic exposures are associated with increased sperm DNA damage, the exact mechanisms by which sperm DNA damage occurs remain unclear (12). Breaks in sperm DNA may be result of compaction errors or defects in repair of DNA breaks from free radicals as well as abnormal nuclear proteins structure. Why some men are more susceptible to developing such damage than others is also unknown. Suganuma et al. (13) have postulated that sperm are susceptible to damage during passage through the male reproductive tract. Indeed, testicular sperm have been suggested to have significantly lower levels of DNA damage compared with ejaculated spermatozoa from the same individuals (14, 15). Conversely, however, testicular sperm have been shown to higher rates of aneuploidy compared with ejaculated sperm from the same individual (16), a finding that could offset the advantage of using testicular sperm for ICSI if performed indiscriminately.

Thus, the optimal treatment options for patients who have previously failed ART cycles using ejaculated sperm with a high proportion of chromatin damage remains controversial. Our study evaluated reproductive outcomes using testicular sperm in oligospermic men who had previously failed to achieve paternity and who had TUNEL-positive ejaculated sperm.

MATERIALS AND METHODS

Study Population

Oligospermic men (sperm concentration <5 million/mL), who, in the absence of identifiable female factor infertility, had previously failed one or more IVF or ICSI cycles performed using ejaculated sperm, and who had decided to undergo microsurgical testicular sperm extraction (TESE) at our academic specialty center between 2008 and 2013, were identified for inclusion in this cohort study. A failed ART cycle was defined as a cycle that did not result in a clinical pregnancy (no heartbeat). Men with a history of obstructive azoospermia, uncorrected varicoceles, testicular trauma, orchiectomy, chemotherapy, or pelvic radiation were excluded.

Per our standard practice, all men undergoing TESE provided an ejaculated semen sample on the day of surgery before their procedure. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed, as described herein, on ejaculated and testicular sperm samples from all men meeting the eligibility criteria for this study. Only patients with >7% TUNEL-positive sperm in ejaculated semen samples were included in the final data analysis.

Surgically retrieved sperm was used for injection of all oocytes in fresh ICSI cycles for testicular sperm retrieval cycles. The medical records of the male patients and their female partners were reviewed to abstract data on demographics, reproductive history, semen parameters, sperm DNA fragmentation, and reproductive outcomes after the use of testicular sperm.

TUNEL Assay

Sperm DNA fragmentation was assessed using the in situ Cell Death Detection Kit with fluorescein isothiocyanate (FITC) (Roche Diagnostics). All assays were performed in duplicate by the same researcher (A.B.), and included one positive and one negative control. Briefly, 10-μL aliquots of each patient sample were smeared on to glass slides and air dried. Each slide was fixed with 1 mL of 4% paraformaldehyde in phosphate-buffered saline (PBS) solution and incubated at room temperature for 1 hour. Slides were washed with ice-cold PBS three times, then permeabilized with TritonX in 0.1% sodium citrate for 5 minutes. Slides were then rewashed with PBS three times then incubated with a mixture of the TUNEL enzyme solution (Roche Diagnostics) containing terminal deoxynucleotidyl transferase, and TUNEL Label Mix (Roche Diagnostics) containing deoxyuridine triphosphate. The slides were individually covered with Parafilm (Ted Pella) and were incubated in a dark, moist chamber at 37°C for 1 hour to allow time for labeling.

After removal, the slides were washed with PBS, and counterstained with Prolong Gold antifade reagent with 4′,6-diamino-2-phenylindole, dihydrochloride (DAPI; Invitrogen) overnight. The slides were then analyzed using an epifluorescence microscope (Fig. 1) fitted with Nomarksi optics (Fig. 2) at ×1,000 magnification. The number of DAPI-positive and FITC-positive cells per high power field were counted. At least four separate fields of view were analyzed for each slide, and at least 200 DAPI-positive cells were counted. The number of FITC-positive cells detected were divided by the number of DAPI-positive cells to calculate the percentage of TUNEL-positive cells containing fragmented DNA.

Data Analysis

The primary outcome measures were [1] the TUNEL-positive proportion of sperm in ejaculated and testicular samples and [2] an intrauterine pregnancy. A paired *t* test was used to compare the differences in TUNEL percentages between ejaculated and testicular sperm both overall and with respect to pregnancy outcomes. Unpaired *t* tests for continuous variables and the chi-square and Fischer's exact tests for categorical variables were used to analyze differences in maternal and paternal age, maternal gravity and parity, number of