Intracytoplasmic sperm injection outcome of ejaculated versus extracted testicular spermatozoa in cryptozoospermic men

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Objective: To compare intracytoplasmic sperm injection (ICSI) outcome of patients with cryptozoospermia after use of ejaculated versus testicular sperm in different cycles of the same patients.
Design: Retrospective cohort study.
Setting: University-affiliated infertility center.
Patient(s): A total of 17 patients with cryptozoospermia who underwent a total of 116 ICSI cycles.
Intervention(s): The patients initially underwent several ICSI cycles using ejaculated sperm (n = 68, 58.6%) that were followed by ICSI cycles using testicular sperm (n = 48, 41.4%).
Main Outcome Measure(s): Fertilization rate, pregnancy rate (PR).
Result(s): There were no significant differences in fertilization rates between the two subgroups. A comparison between testicular sperm extraction (TESE) versus ejaculated sperm cycles revealed significantly higher implantation rate (20.7% vs. 5.7%), higher PR (42.5% vs. 15.1%), and higher take home baby rate (27.5% vs. 9.4%). A multivariable logistic regression analysis showed three significant predictors for pregnancy, namely the use of testicular sperm (odds ratio [OR] 5.1, 95% confidence interval [95% CI] 1.8–14.8), use of motile sperm (OR 12.9, 95% CI 2.1–79.1), and female age (OR 0.83, 95% CI 0.7–0.9).
Conclusion(s): Testicular sperm extraction is justified in patients with cryptozoospermia who fail to conceive by ICSI using ejaculated spermatozoa, as it offers higher PR. (Fertil Steril® 2013;99:1867–71. ©2013 by American Society for Reproductive Medicine.)
Key Words: Cryptozoospermia, ejaculated sperm, ICSI outcome, male factor infertility, testicular sperm extraction (TESE)

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Cryptozoospermia is defined by the World Health Organization as the constant presence of isolated sperm cells in the ejaculate that can be found after an extended microscopic search (1). The use of extended sperm preparation enables the identification of these isolated spermatozoa, which may be subsequently used for intracytoplasmic sperm injection (ICSI) (2–4). The introduction of ICSI (5) has revolutionized the treatment of severe male factor infertility, as the only absolute prerequisite for successful ICSI is the presence of one spermatozoon per oocyte.

One central question concerning ICSI is whether the type or the extent of sperm impairment has any influence on the outcome. Several centers have not found that impaired sperm parameters affect fertilization and pregnancy outcome after ICSI (6, 7). In contrast, our group found that an extremely limited number of sperm in the initial ejaculate resulted in compromised fertilization and pregnancy rates (PR) (8). This has been attributed to contaminating cells in the ejaculate (9) and exposure to oxygen-free radicals (10). It was also postulated that sperm may be susceptible to damage during passage through the male genital tract (11). Therefore, when fertilization fails or implantation is not achieved these patients are candidates for testicular sperm extraction (TESE), as all other patients with nonobstructive azoospermia

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based on the assumption that spermatozoa retrieved directly from the testicle may provide healthier gametes capable of generating more viable embryos (12).

Because in cryptozoospermia both ejaculated and testicular spermatozoa are available, it is uncertain which is preferable for the ICSI procedure. Few studies compared the fertility outcome of ejaculated versus fresh testicular sperm cells in the same patients. Weissman et al. (13) showed in four couples with severe oligoteratoasthenozoospermia syndrome who, with the use of ejaculated spermatozoa for ICSI resulted in poor embryo quality and repeated implantation failure, whereas the use of fresh testicular sperm cells resulted in embryo implantation and ongoing pregnancies. Bendikson et al. (9) compared the outcome of ICSI cycles using either fresh ejaculated or testicular sperm in 16 men diagnosed with virtual azoospermia, showing a trend favoring testicular sperm. Hauser et al. (4) compared the outcome of ICSI cycles using either ejaculated or testicular sperm in 13 patients defined as having either virtual azoospermia (n = 5) or cryptozoospermia (n = 8), showing that fresh testicular sperm yielded better implantation rates than both frozen testicular and ejaculated sperm.

Because the data in the literature regarding ICSI outcome of ejaculated versus extracted testicular spermatozoa in men with cryptozoospermia are scarce, the aim of our current study is to compare the ICSI of patients with cryptozoospermia after the use of ejaculated versus testicular sperm in different cycles of the same patients.

MATERIALS AND METHODS

Selection of Patients

All patients with cryptozoospermia who underwent ICSI cycles with either ejaculated or testicular sperm between January 2010 and December 2011 in our IVF unit were candidates for inclusion in this study. Seventeen couples met the study criteria after exclusion of cases with virtual azoospermia and/or poor ovarian response and in whom only ejaculated sperm was used. The current study was approved by the local Institutional Review Board committee.

Testicular retrieval procedures are invasive and harbor several potential complications such as vascular injury and inflammatory changes (14). Therefore, our policy in patients with cryptozoospermia is to initially use ejaculated sperm cells for ICSI. The data in the literature regarding ICSI outcome of ejaculated versus extracted testicular spermatozoa in men with cryptozoospermia are scarce. Therefore, there is neither consensus nor uniform criteria regarding the exact number of failed ICSI ejaculated sperm cycles in which an individual couple should proceed to ICSI-TESE cycle. Whenever fertilization, embryo development and/or PRs were poor after several ICSI cycles using ejaculated sperm, these patients underwent a TESE procedure. For each couple we individualized this decision according to several parameters (e.g., the outcome of the previous ICSI ejaculated sperm cycles, female age, ovarian response, and the couple's preference). The TESE procedure was performed on the day of ovum pickup, thus fresh testicular sperm cells were initially used.

Methodology of the TESE Procedure

The surgical technique of testicular sperm retrieval in patients with cryptozoospermia was identical to the one described for patients with nonobstructive azoospermia by our group (15). Briefly, after stabilization of the testicle, a small incision in the testicle's midportion was performed, cutting through the scrotal skin, tunica vaginalis, and albuginea. A substantial piece of the extruding testicular tissue was cut with small scissors, washed with medium to remove blood traces, and placed in a Petri dish containing 1–3 mL Earle's balanced salt solution (EBSS) with heparin (GIBCO). Testicular tissue was vigorously fragmented and minced using two glass slides and immediately examined under the inverted microscope for the presence of spermatozoa in a wet preparation. Once spermatozoa were found, the surgical procedure was terminated. If spermatozoa were not observed, up to three biopsies were taken, from different areas of the same testicle and also from the contralateral one. Specimen was sent for histologic analysis. After vigorous tissue shredding, human tubal fluid (HTF)– *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)–albumin medium supplemented with 7.5% synthetic serum was added to the suspension obtained and incubated in 6-mL Falcon tubes, for 2 hours (5% CO_2 in air) at 37°C. The overlying pellet was then collected and centrifuged (300 × *g* for 10 minutes). The remaining pellet was isolated, processed, and finally examined in multiple droplets under oil. Also, the remains of the underlying pellet were processed and examined in multiple droplets, as described previously. Excess testicular material in cases where sperm were detected was frozen for subsequent thawed sperm ICSI cycles.

Ovarian Stimulation

Ovarian stimulation was performed using the routine long protocol of pituitary suppression followed by ovarian stimulation. Oocytes were retrieved by vaginal ultrasound-guided follicular puncture. Embryo transfer was performed on day 2 or 3 after oocyte retrieval. The best embryos were selected for transfer. Supernumerary top quality embryos were frozen and the remainder were followed for blastocyst formation. After ET, all patients received luteal support, including IM injections of hCG, or 600 mg of vaginally administered micronized P (Utrogestan; Laboratories Piette International S.A.). Clinical pregnancy was defined as a visible sac on the fifth gestational week.

Statistical Methods

Descriptive parameters were expressed as mean ± SD. Frequencies were presented as percentages. Differences in means between two variables were calculated using Student's *t*-test, whereas χ^2 test was used for comparison of proportions. Comparison of mean values among the three subgroups was performed by using analysis of variance test. Calculations were performed by using SPSS software (version 11; SPSS, Inc.). *P* values of less than .05 were considered statistically significant.