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Influence of multiple transrectal electroejaculations on semen parameters and intracytoplasmic sperm injection outcome

Simone Giulini, M.D.,[a] *Francesco Pesce, M.D.,*[b,e] *Igael Madgar, M.D.,*[c] *Tiziana Marsella, B.D.,*[a] *Annibale Volpe, M.D.,*[a] *Domenico de Aloysio, M.D.,*[d] *and Cesare Battaglia, M.D., Ph.D.*[d]

University of Modena and Reggio Emilia, Modena, Italy; The Chaim Medical Center, Tel Hashomer, Israel; and University of Bologna, Bologna, Italy

Objective: To compare semen parameters and intracytoplasmic sperm injection (ICSI) outcome in spinal cord–injured subjects who underwent single (group 1) or multiple (group 2) electroejaculations before ICSI.

Design: Prospective, randomized, controlled study.

Setting: Department of gynecology, obstetrics, and pediatric science in a reproductive medicine unit at a major Italian university.

Patient(s): Thirty-four healthy women with a male partner with SCI who were seeking assisted reproduction services.

Intervention(s): Transrectal electroejaculation, controlled ovarian hyperstimulation, and ICSI.

Main Outcome Measure(s): Sperm concentration, morphology, and motility and fertilization and pregnancy rates after ICSI.

Result(s): Sperm was successfully retrieved in 94.1% of cases. In male subjects who underwent multiple electroejaculations, statistically significant improvements in sperm concentration and total sperm motility rate were observed. The overall fertilization rate was 63.6%. The number of oocytes retrieved and injected was comparable between the two groups. A total of nine clinical pregnancies were achieved. The pregnancy rate was statistically significantly higher in group 2 (n = 6/16; 37.5%) than in group 1 (n = 3/16; 18.75%).

Conclusion(s): These data suggest that multiple electroejaculation has a positive effect on semen parameters and ICSI outcome. (Fertil Steril® 2004;82:200–4. ©2004 by American Society for Reproductive Medicine.)

Key Words: Electroejaculation, ICSI, semen parameters, spinal cord injury

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Reprint requests: Simone Giulini, M.D., Department of Gynaecological, Obstetric and Paediatric Sciences, University of Modena and Reggio Emilia, L.go del Pozzo 71, 41100 Modena, Italy (FAX: 39-59-4224349; E-mail: sgiulini@unimore.it).

[a] Department of Gynaecological, Obstetric and Paediatric Sciences, University of Modena and Reggio Emilia.

[b] Department of Urology, University of Modena and Reggio Emilia.

[c] Department of Urology, The Chaim Medical Center.

[d] III Obstetrics and Gynecology Department, University of Bologna.

[e] Present address: Hospital Policlinico Rossi, Verona, Italy.

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In men, infertility is one of the major consequences of spinal chord injury (SCI). Although reflex erections are common, ejaculation occurs rarely. It is estimated that <5% of men with SCI can procreate without medical intervention (1).

Transrectal electroejaculation (TREE) is an established method for semen retrieval in anejaculatory men, with recovery rates ranging from 70% to >90% (2–4). However, the quality of semen obtained by TREE is almost always poor. Electroejaculates are characterized by small volumes, variable sperm concentrations, and poor sperm quality (5). Furthermore, TREE is associated with decreased sperm viability, poor bovine cervical mucus penetration, suboptimal hamster egg penetration, and decreased motility longevity (6).

These abnormalities have been attributed to many factors, including stasis of accessory sex organ fluids, defective scrotal temperature regulation, recurrent urinary tract infections, complications associated with bladder management, chronic medications, sperm autoimmunity, generation of excessive reactive oxygen species, abnormal spermatogenesis, dysregulation of the hypothalamic-pituitary-testicular axis, retrograde ejaculation, urine contamination during assisted ejaculation, detrimental effect of electrical current, and poor overall health of

the subject (6–11). Although each of these factors could contribute to the overall deterioration in semen quality, the mechanisms responsible for semen abnormalities in SCI men are not well defined.

The development of assisted reproduction techniques has enabled these patients to achieve a pregnancy with their partners. However, until the advent of intracytoplasmic sperm injection (ICSI), pregnancy rates were very low (4, 5, 12, 13). Some investigators have reported improvements in semen parameters after repeated electroejaculations (2, 14, 15), but others have not observed this association (6, 16). A large part of previous studies were retrospective, had small sample sizes, and lacked adequate control groups for comparison.

Thus, to test the hypothesis that SCI males who undergo repeated TREE before ICSI would have improvements in semen quality and subsequently more favorable ICSI outcomes, we designed a randomized, prospective, controlled intervention. The aim of the present study was to compare sperm parameters and ICSI outcome in SCI subjects who underwent single or multiple TREE before ICSI.

MATERIALS AND METHODS

The study protocol was approved by the Departmental Ethics Committee of the University of Modena. Written informed consent was obtained from all subjects before the beginning of study-related procedures.

Subjects

Thirty-four couples seeking assisted reproductive services were enrolled and studied. The presenting cause of infertility was anejaculation secondary to SCI in the male partner. Six to 12 months before study enrollment, couples underwent a standard infertility evaluation, including an initial semen analysis obtained by TREE, to exclude those with female factor or other organic causes of infertility, including testicular dysfunction. Couples in whom the female partner was aged ≥38 years were also excluded from participation. All male subjects were free from urinary tract infections. After the initial infertility evaluation, the male partner was randomly assigned to undergo a single TREE (group 1, n = 18) or to undergo multiple (basal, after 1 month and after 3 months) TREEs (group 2, n = 16) before ICSI.

Sperm Retrieval

Subjects received 7 to 10 days of oral antibiotics (doxycycline, 0.1 mg/d) before each procedure to protect the retrograde ejaculate from bacteria and white blood cell contamination. Urine was alkalyzed with 650 mg/d of oral sodium bicarbonate administered 48 hours before each procedure. Before the electrostimulation, the urinary bladder was catheterized and washed with 40 mL of fresh *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid–buffered culture medium. Subjects with a history of autonomic dysreflexia and/or with high spinal cord lesion were treated 15 minutes before the procedure with 10 to 20 mg of sublingual nifedipine. Those with residual sensation underwent stimulation under general anesthesia.

The patients were placed in the lateral decubitus position, and after proctoscopic examination, TREE was performed with a 5-cm-diameter Saeger rectal probe placed against the prostate and seminal vesicles, as described by Bennet et al. (17). Blood pressure and rectal temperature (sensor in the rectal probe) were monitored during the procedure. Multiple electrical stimulations ranging from 5 to 20 V and lasting for approximately 2 seconds were administered. The urethra was manually milked into a sterile container both during and upon completion of electrostimulation to obtain the maximum volume of antegrade semen. After electroejaculation, a bladder catheterization was routinely performed for collection of retrogade specimens.

Semen parameters were assessed with a Makler counting chamber used according to the World Health Organization guidelines (18). The percentage of motile sperm was determined on ≥200 sperm. Nonmotile sperm were assigned a score of zero. Motile sperm were graded as 1 (no forward progression), 2 (forward progression), or 3 (rapid, linear forward progression). Sperm morphology was evaluated by Papanicolaou staining and classified according to World Health Organization criteria (18).

Preparation of Sperm for ICSI

After collection, spermatozoa were washed by diluting in 2 volumes of culture medium and processed by centrifuge at 500 × *g* for 8–10 minutes at room temperature. The sperm pellet was resuspended into culture medium, and equal volumes (1 mL) were loaded onto two or three Pure Sperm (Nidacon AB, Gothenburg, Sweden) density gradients (95%, 70%, and 50%), which were then processed by centrifuge at 450 × *g* for 20 minutes at room temperature. After centrifugation, the 95% layer was aspirated in and out of a pipette and passed through the other two layers to resuspend the sperm before removing it to another tube without disturbing the interface. The recovered 95% Pure Sperm layer was diluted with 2 mL of medium, and the sample was processed by centrifuge at 500 × *g* for 8–10 minutes at room temperature. The supernatant was removed, and the sperm pellet was resuspended in culture medium for ICSI.

Ovarian Stimulation

Briefly, controlled ovarian hyperstimulation was achieved by an IM injection on day 20 of the cycle of gonadotropin-releasing hormone agonist (Decapeptyl 3.75; Ipsen, Milan, Italy) and pure FSH (pFSH; Metrodin HP; Serono, Rome, Italy) administered IM (225 IU/d during the first 3 days of stimulation and 150 IU/d thereafter) after pituitary desensitization (plasma estradiol <100 pmol/L, no follicles sized >5 mm in diameter, and endometrial thickness <5 mm on transvaginal ultrasound examination). When more than three

follicles of >17 mm in diameter were present, pFSH was withdrawn, and 10,000 IU of hCG (Profasi; Serono, Rome, Italy) were administered IM.

Ultrasonographic transvaginal oocyte recovery was performed 35 hours after hCG injection.

The ICSI procedure was performed as described by Palermo et al. (19, 20). Embryo transfer was performed with a Frydman catheter (SCS International, Genoa, Italy), approximately 72 hours after oocyte retrieval. Between two (in women <35 years) and three (in women ≥35 years) embryos were replaced at the 8-cell to 12-cell stage. The remaining cleaved embryos with <20% fragmentation were allocated to a cryopreservation protocol.

Vaginal progesterone (Esolut Crema; Angelini, Rome, Italy) was prescribed daily as luteal support, beginning on the day of embryo transfer and continuing until the β-hCG assay was performed. Subjects with clinical pregnancy (ultrasonographic evidence of embryonic heart activity) were followed up until after delivery.

Statistical Analysis

A statistical analysis (SPSS software; SPSS Inc., Chicago, IL) was performed by using the unpaired Student's *t*-test and one-way analysis of variance. $P \leq .05$ was considered to be the limit of statistical significance. Data are presented as mean ± SD unless otherwise indicated.

RESULTS

Electroejaculations were successfully completed in 32 of 34 patients (94.1%). Ejaculates could not be obtained from two subjects in group 1. These subjects were excluded from the analysis and referred for testicular sperm aspiration (21). The remaining 32 couples underwent TREE, followed by ICSI.

The mean female age was 29.4 ± 5.1 (range 21–37 years) years. The mean age of the men was 34.9 ± 6.3 (range 28–46 years) years. The level of SCI ranged from cervical vertebra 6 to lumbar vertebra 1.

During TREE, the mean number of electrical stimulations delivered per session was 13.7 ± 5.8 (range 4–25), at a mean voltage of 13.7 ± 4.1 V (range, 5–20 V). No complications were observed during or after the procedure(s). In basal condition, the mean antegrade volume obtained was similar between group 1 (1.4 ± 0.6 mL) and group 2 (1.3 ± 0.8 mL). In addition, in group 2 patients, no differences were observed by comparing the basal antegrade sperm volume with 1-month electroejaculation (1.4 ± 0.4 mL) and 3-month electroejaculation (1.6 ± 0.5 mL) values.

Furthermore, the rate of normal sperm morphology was not significantly different (group 1 28% vs. group 2 basal, 26%; or vs. 1-month electroejaculation, 32%; and vs. 3-month electroejaculation, 35%) between the groups. However, in subjects who underwent multiple TREEs, the mean sperm concentrations at 1 month (27.2 ± 7.6 × 10^6/mL) and 3 months (34.0 ± 9.5 × 10^6/mL) were significantly ($P<.001$) higher when compared with the basal value (18.0 ± 4.8 × 10^6/mL) and the concentration in the single-TREE group (18.7 ± 5.9 × 10^6/mL; Fig. 1). The mean rate of total sperm motility increased significantly ($P<.001$) after 1-month (33%) and 3-month (40%) electroejaculations in comparison with both group 1 (15%) and group 2 (13%) basal values (Fig. 2). This improvement was primarily due to the increased proportion of grade 1 and grade 2 sperm, 30% ($P<.001$) after 1 month and 32% ($P<.001$) after 3 months,

FIGURE 1

Sperm count (× 10^6/mL) in group 1 and group 2. *Blue bars*, group 1; *red bars*, group 2. *$P<.001$, 1-month electroejaculation and 3-month electroejaculation vs. both group 1 and basal group 2.

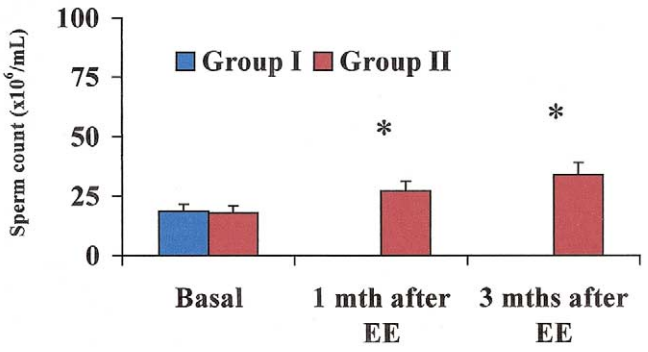

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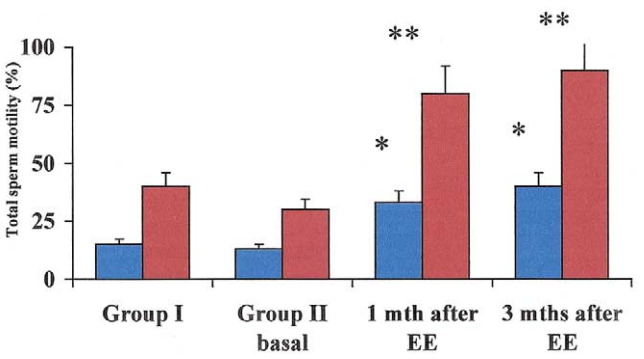
FIGURE 2

Sperm motility in group 1 and group 2. *Blue bars*, pretreatment; *red bars*, after Pure Sperm treatment. *$P<.001$, 1-month electroejaculation and 3-month electroejaculation vs. both group 1 and basal group 2 (pretreatment). **$P<.001$, 1-month electroejaculation and 3-month electroejaculation vs. both group 1 and basal group 2 (after Pure Sperm treatment).



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compared with 13% in group 1 and 11% at the basal electroejaculation of group 2. The numbers of sperm with rapid, linear forward progressive motility remained low and did not change significantly.

After Pure Sperm density gradient centrifugation, the mean rate of total sperm motility increased significantly after 1 month (80%; $P<.001$); and 3 months (90%; $P<.001$), compared with both group 1 (40%) and basal values of group 2 (30%; Fig. 2). Even after sperm preparation, this significant improvement was due to the increase of grade 1 and grade 2 sperm motility after 1-month electroejaculation (70%; $P<.001$), and 3-month (75%; $P<.001$) electroejaculation, in comparison with both the group 1 (35%) and basal values of group 2 (25%).

The mean age of female partners was similar between the two groups (group 1 = 30.5 ± 5.2 vs. group 2 = 29.1 ± 4.9 years). No statistical differences were found in terms of days of ovarian stimulation (group 1 = 11.4 ± 2.1 vs. group 2 = 10.2 ± 2.4) and number of pFSH ampules used (group 1 = 24.7 ± 4.8 vs. group 2 = 22.7 ± 6.8). A total of 346 oocytes were retrieved, and 267 were injected, resulting in a fertilization rate (two-pronuclear embryos) of 63.6%. Comparing the two groups, we did not find any significant difference in terms of retrieved oocytes (group 1 = 164 vs. group 2 = 182), injected oocytes (group 1 = 131 vs. group 2 = 136), and rate of fertilization (group 1 = 64.1% vs. group 2 = 63.1%).

The number of replaced embryos (range 2–3) was similar (group 1 = 2.4 ± 0.2 vs. group 2 = 2.5 ± 0.3) among the groups. The pregnancy rate per patient was significantly higher ($P<.001$) in group 2 (n = 6/16; 37.5%) than in group 1 (n = 3/16; 18.75%). All clinical pregnancies were confirmed by ultrasound scanning, and five singleton, three twin, and one triplet pregnancies were detected.

DISCUSSION

In anejaculatory SCI patients, electroejaculation is the most efficient method of sperm retrieval (2–4).

In our series, the study populations had spinal-cord lesions at different levels (from cervical vertebra 6 to lumbar vertebra 1). To be sure of obtaining ejaculation in almost all patients and of having comparable results, TREE has been performed as a first-line attempt to obtain sperm in all cases. In agreement with previous reports (2–4), the sperm retrieval was successfully performed in 94.1% of the cases, and no serious side effects (e.g., autonomic dysreflexia) occurred.

Despite a high success rate in sperm recovery by electroejaculation in men with SCI, the poor-quality semen remain a problem. The loss of sperm quality after SCI may be due to anejaculation since injury. Furthermore, the stasis of sperm and accessory sex organ fluids in reproductive tract is also associated with defective scrotal temperature regulation. Men with chronic SCI who sit in wheelchairs have scrotal temperatures about 1°C higher than do able-bodied men (22).

The quality of semen that is produced by electroejaculation from men with chronic SCI is similar to that seen with epididymal necrospermia, a rare condition affecting ≤1 in 200 infertile men (15): sperm motility of <20% and sperm viability (assessed by die exclusion) of <30%. In men with necrospermia, the electron-microscopic examination of ejaculated spermatozoa shows severe degenerative changes. However, sperm in the lumen of seminiferous tubules and in the corpus epididymis appears normal. In this particular condition, frequent ejaculation was shown to improve sperm motility, viability, and appearance on electron microscopy. On the basis of these findings, it was suggested that the basic defect is caused by the epididymal sperm storage (23).

In men with SCI because of neurological dysfunction, the contents of the vas fail to clear completely. The vas and the seminal vesicles may become packed with sperm, secretions, leukocytes, and cells that are involved in removal of aging sperm. Thus, this stasis can be removed with the first electroejaculation procedure. Subsequent frequent electroejaculations would clear the vasa, cauda epididymides, and seminal vesicles, allowing younger sperm to later appear in the ejaculate. This possibility is also supported by reports that highly motile sperm concentrations can be obtained by aspirating or flushing the proximal end of the vas in men with chronic SCI (23, 24).

In this study, to analyze the possible negative effects related with sperm and sex organ fluid stasis, we randomly assigned SCI patients to a single electroejaculation or to multiple electroejaculations before ICSI. In group 1 we performed a single TREE procedure the same day of the ovum pickup, whereas in group 2 after a basal electroejaculation, TREE was repeated after 1 month to completely clear the vas and the seminal vesicles and was performed again after 3 months, on the same day of ovum pickup. Our findings, in agreement with those of other reports (2, 14, 15), show that multiple electroejaculation significantly increase the sperm count and percentage of total motility.

However, other investigators did not find this correlation (6, 14). Regarding sperm morphology, it has been reported that the high rate of teratozoospermia present in the electroejaculation may contribute to the decreased fertility (5, 16). In our study, the mean rate of sperm with normal morphology was similar between the two groups, and we did not find any improvement after multiple electroejaculations.

In our series, despite the better semen parameters obtained in group 2 after repeated electroejaculations before ICSI, the fertilization rate and the mean number of transferred embryos were not different among the two groups. However, in group 2 we found a significantly higher pregnancy rate per patient. We postulated that the better semen parameters obtained after multiple electroejaculation by re-

ducing sperm aging and possible consequent DNA damage may have some positive effects on subsequent embryo quality. Moreover, it has been widely recognized that the pregnancy rate is strictly correlated to the quality of transferred embryos. Further studies on sperm obtained by electroejaculations in SCI men are necessary to verify the effects of sperm aging on DNA compromise.

In conclusion, in male infertility caused by SCI, repeated electroejaculations seem to have positive effects on semen parameters and ICSI cycles outcome. Further, more extensive, prospective randomized studies are necessary for better understanding the relationship between sperm aging and fertility in SCI men.

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