Effect of seminal leukocytes on in vitro fertilization and intracytoplasmic sperm injection outcomes

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Objective: To investigate the influence of seminal leukocytes on conventional IVF and intracytoplasmic sperm injection (ICSI) outcomes, using a flow cytometry method.
Design: Prospective study.
Setting: Tertiary infertility center and research institute.
Patient(s): One hundred sixty-four couples undergoing conventional IVF or ICSI.
Intervention(s): Seminal leukocytes were counted by flow cytometry.
Main Outcome Measure(s): Correlation between seminal leukocytes concentration and reproductive outcomes in IVF and ICSI cycles.
Result(s): The median number of oocytes retrieved, the fertilization and cleavage rate, the median number and grade of embryos transferred, the median number of good-quality embryos transferred, and the median percentage of good-quality embryos from total embryos transferred, in leukocytospermic and non-leukocytospermic patients were not statistically different after either IVF or ICSI. Similarly, there were no significant differences between the two groups for implantation rate and clinical pregnancy rate. Multivariate logistic regression analysis showed that the reproductive outcomes were not influenced by adjustment for female age, infertility diagnosis, number of previous attempts, treatment protocol (GnRH agonist or antagonist), assisted reproduction procedure (IVF or ICSI), and leukocytospermia. By profiling the proper Poisson regression models, no leukocytospermia cut-off value was able to identify the subjects at risk for oocyte fertilization or embryo cleavage failure.
Conclusion(s): Using a flow cytometry method, we demonstrated that leukocytospermia does not significantly influence IVF or ICSI outcomes. The same results were obtained by using lower or higher cut-off values for leukocytospermia (from 0.2 to 2 $\times$ 10^6/mL). (Fertil Steril® 2015;104: 87–93. ©2015 by American Society for Reproductive Medicine.)
Key Words: Assisted reproductive technology, flow cytometry, ICSI, IVF, leukocytospermia

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Leukocytospermia is frequently detected during the semen analysis performed before starting an assisted reproductive technology (ART) cycle (1–4). In this event, microbiological tests and, if necessary, andrologic evaluation and adequate treatment should be performed before progressing to the procedure (5). However, in some cases leukocytospermia represents an unexpected finding on the day of oocyte pick-up (6). In this instance two management options can be considered: either to progress to oocyte insemination, performing intracytoplasmic sperm injection (ICSI) that reduces to a minimum the contact between the egg and the sperm, or to put off the procedure and cryopreserve the oocytes. The second option is less favorable because not all fertility clinics have extensive experience in oocyte cryopreservation (7). Furthermore, the pregnancy rate from frozen–thawed oocytes is still significantly lower than that from fresh cells (8). Unfortunately, literature data are unclear for providing definitive

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guidance for the management of these cases. In fact, several studies are available, but they provide conflicting data on the effect of leukocytospermia on ART outcomes. Some studies have suggested that leukocytospermia negatively affects the outcome of both conventional IVF and ICSI (9–15). Conversely, in other studies leukocytospermia has been reported to have no negative influence on fertilization or pregnancy rates after IVF, ICSI, or intracytoplasmic morphologically selected sperm injection (16–22).

Under wet mount microscopy, leukocytes and immature germ cells appear quite similar. A number of methods have been proposed to differentiate the two types of cells (1). Most studies have used methods based on the peroxidase activity for detecting seminal leukocytes, and this might explain the controversial results obtained. In fact, the sensitivity of the peroxidase test compared with that of more reliable methods is low, ranging from 47% to 60% (23–26), even though the last World Health Organization (WHO) manual for the semen examination still recommends this method for the initial screening of leukocytospermia (27). Specifically, it has been demonstrated that this procedure has a low counting precision, especially at low concentration of leukocytes (28). In fact, Cooper and Hellenkemper (28) have calculated that, at the recommended 1 + 9 dilution, for semen containing the consensus limit of 1×10^6 leukocytes per milliliter, the sampling error is 22% (95% confidence interval 1,220,000–780,000 cells per milliliter). Moreover, they have shown that, by using the WHO-recommended procedure, only samples containing 20×10^6 round cells per milliliter would achieve a counting error of 5% (28).

We have shown that flow cytometry offers the possibility of a simple, rapid, and accurate assessment of several semen parameters, including a highly precise count of seminal leukocytes (29). Using this method, we have also demonstrated that the final concentration of leukocyte in prepared semen samples is significantly different depending on the use of swim-up or density-gradient centrifugation (30).

The aim of this prospective study was to assess the effect of leukocytospermia on IVF and ICSI outcomes, using flow cytometry for semen leukocytes count.

MATERIALS AND METHODS

Study Population and Semen Analysis

Couples undergoing ART procedure at the Assisted Reproduction Unit of the Institute for Maternal and Child Health, IRCCS "Burlo Garofolo," Trieste, Italy, were included in the study. The study was approved by the institutional review board. Informed consent was obtained from all subjects. All patients were asymptomatic for genitourinary infections and had been screened for chlamydia, mycoplasma, and other bacterial infections. Semen samples were evaluated on the day of oocyte pick-up. Semen analysis was performed according to WHO criteria (27). Specimens were collected into a sterile container by masturbation after 3 to 4 days of sexual abstinence. After liquefaction, semen was evaluated for volume, viscosity, sperm concentration, progressive motility, and round cells concentration (10^6/mL). Semen samples were processed by using the swim-up method either for IVF or ICSI (30).

Leukocyte Count

Leukocyte count was carried out using a standard peroxidase test, as described in the WHO laboratory manual (27), and using flow cytometry (29). A multiparameter flow cytometric analysis was carried out, as previously described (29). Briefly, 100 μL of fresh semen specimen was stained with 2 μL of a 10-μM solution of Syto 16 (Molecular Probes), 10 μL of 7-amino-actinomycin-D (Via-Probe, BD Pharmingen), and 10 μL of allophycocyanin-conjugated mouse monoclonal anti-CD45 antibody (Caltag Laboratories). One hundred microliters of Flow-Count fluorospheres (Beckmann-Coulter) were added to perform the absolute count. After 20 minutes of incubation at room temperature and in the dark, 1 mL of phosphate-buffered saline was added to each tube, and samples were analysed by flow cytometry.

ART Procedures

All patients were treated with a long GnRH analogue suppression protocol or a flexible GnRH antagonist protocol with oral contraceptive pill pretreatment. Recombinant FSH (150–225 IU/d) was started when pituitary down-regulation was established or from day 2 of the cycle, in the GnRH agonist and antagonist protocol, respectively. The recombinant FSH dose was adjusted and/or highly purified menotropin was added, after 4 days of stimulation, depending on the ovarian response, as assessed by serum E_2 levels and ultrasound. Human chorionic gonadotropin (5,000 or 10,000 IU) was administered when at least two leading follicles reached a mean diameter of 18 mm. Transvaginal oocyte pick-up was carried out 36 hours later. Two or three embryos were transferred on day 3 after oocyte pick-up. The luteal phase was supported by vaginal administration of micronized P (600 mg/d). Embryos were scored as previously described (31). Serum hCG levels were measured 14 days after ET and, if positive, an ultrasound scan was scheduled 2 weeks later to assess the number and status of the implanted embryos.

Statistical Analysis

The data were analysed using R software (32). To assess differences between counts data the nonparametric Mann-Whitney *U* test was addressed. Fisher exact test was used to assess association in contingency tables. Generalized linear models (33) were used in counts (Poisson regression) and proportion (logistic regression) data to address multivariate analysis, correcting for over-dispersion when required (34). Generalized models are reported according to the Wilkinson and Rogers notation (35). In all analyses, statistical significance was assumed at a 0.05 α level.

RESULTS

ART Outcomes

A total of 164 couples were recruited. Fifty-nine couples underwent IVF treatment, whereas 105 had ICSI treatment.