In Vitro Fertilization and Intracytoplasmic Sperm Injection for Couples with Unexplained Infertility After Failed Direct Intraperitoneal Insemination

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Submitted: March 3, 2000
Accepted: April 21, 2000

Purpose: The objective was to determine the optimal insemination technique in patients undergoing in vitro fertilization (IVF) after failed direct intraperitoneal insemination (DIPI) and the outcome of intracytoplasmic sperm injection (ICSI) in such cases.

Methods: In case–control studies, 53 couples with unexplained infertility who underwent IVF after four failed DIPI cycles were compared with 75 couples with tubal or endometriosis infertility as controls. Thirty couples with unexplained infertility after failing to conceive with DIPI and conventional IVF who underwent ICSI and 58 couples with male-factor infertility as controls also were compared. Fertilization cleavage, embryo quality, implantation, and pregnancy were compared after IVF and after ICSI.

Results: There was a significant difference in fertilization rates after IVF between cases of unexplained infertility after failing to conceive with DIPI (40.4%) and patients with tubal or endometriosis infertility (67.9%). There also was a significant difference in total fertilization failure rates between the two groups (30.4% and 3.9%, respectively). There was a slight but significant difference in numbers of fertilized oocytes after ICSI between patients with low fertilization rate undergoing IVF after failing to conceive DIPI (85.8%) and patients with male factor (90.4%). Total fertilization failure was not observed in these cases.

Conclusions: Couples with unexplained infertility after failing to conceive with DIPI show a failed fertilization or a low fertilization rate after IVF. However, they demonstrated a good chance of becoming pregnant after subsequent ICSI, even with statistically significant difference in fertilization rate as compared with male-factor cases.

KEY WORDS: Intracytoplasmic sperm injection; unexplained infertility; in vitro fertilization; direct intraperitoneal insemination; failed fertilization.

INTRODUCTION

The prevalence of infertile couples without any identifiable cause (unexplained infertility) has been reported to vary between 1% and 37% (1). Intrauterine insemination (IUI), especially direct intraperitoneal insemination (DIPI), with previously reported pregnancy rates of 22.7% (2), 23.0% (3), or 35.6% (4), appears to offer the advantage of being a relatively noninvasive way of dealing with unexplained infertility and is well worth trying before moving on to more invasive and costly approaches such as gamete intrafallopian transfer (GIFT) or in vitro fertilization (IVF). Gurgan et al. (5) and Ruiz et al. (6) reported that there was a higher incidence of total fertilization failure events in couples with unexplained infertility. However, the prognosis of couples failing to conceive with superovulation and DIPI is unknown. Furthermore, it remains to be defined whether couples failing to conceive with superovulation and DIPI represent a less favorable subset of patients for subsequent treatment with more sophisticated means of assisted reproduction. The present study was designed to determine the prognosis after IVF and subsequent ICSI in couples with unexplained infertility failing to conceive with superovulation and DIPI.
MATERIALS AND METHODS

Patient Selection

The patients were divided into four groups according to the underlying indication for IVF or ICSI. The first IVF group consisted of 53 patients with unexplained infertility who had failed to conceive with four to six episodes of superovulation and DIPI. The second group consisted of 75 patients undergoing IVF because of endometriosis or a tubal factor. The third group consisted of 30 patients undergoing ICSI because of failed fertilization or a low fertilization rate in the first group. The fourth group consisted of 58 patients undergoing ICSI because of severe male factor problems according to the criteria of the World Health Organization (WHO) (7). Unexplained infertility was defined as a normal semen analysis according to the criteria of the WHO, including the absence of antisperm antibodies, and postcoital test. Endometriosis was diagnosed by laparoscopy or laparotomy.

Ovarian Stimulation and Oocyte Collection

Ovarian stimulation was carried out by a desensitizing protocol using the gonadotropin-releasing hormone agonist (GnRHa) buserelin acetate (Suprecur; Hoechst Japan) in combination with human menopausal gonadotropin (hMG) (Humegon; Organon, Japan; or Pergogreen; Seronol Japan). The response to stimulation was determined by serial estradiol determination and ovarian ultrasonograms. When the leading follicle achieved a diameter of 18 mm, human chorionic gonadotropin (hCG) (10,000 IU) was administered and transvaginal ultrasound-guided oocyte retrieval was carried out 36 hr later. hCG injection (1500 IU) on days 2 and 4 and intravaginal micronized progesterone (400 mg/day) were given for luteal support.

Preparation of Sperm

Semen samples were allowed to liquefy for 20 min, prepared with the standard swim-up technique for IVF, and processed through discontinuous Percoll gradients (40% and 90%) for ICSI. Percolled sperm pellets were washed twice and resuspended in HEPES buffered human tubal fluid (modified-HTF, Irvine Scientific, CA) before use.

Preparation of Oocytes

Oocytes were rinsed thoroughly and kept in HTF with 10% heat-inactivated maternal serum [insemination medium, (IM)] for up to 5 hr at 37°C under 5% CO₂ in air. For IVF, fertilized oocytes were transferred to growth medium consisting of HTF with 15% heat-inactivated maternal serum. For preparation of ICSI, the cumulus was removed by repeated aspiration into glass pipettes in modified-HTF with 0.025% hyaluronidase type VIII (Sigma Chemical Co.). Denuded oocytes were thoroughly rinsed and returned to the IM. Only metaphase II oocytes were used for ICSI.

Intracytoplasmic Sperm Injection

The ICSI procedure was performed according to the method described by Kimura and Yanagimachi (8) with minor modifications. Briefly, several piezo-pulses (intensity 2, speed 2) were administered to advance the pipette until its tip passed through the zona pellucida. The pipette then was advanced quickly and manually through the ooplasm until it reached one third of the distance to the opposite side of the cortex of the oocyte. The oolemma was broken (as confirmed by its relaxation) using a light negative pressure without piezo-pulses.

The cover (10 mm in depth) of a plastic dish (60 × 15 mm) (Falcon Plastics, Oxford) was used as a microinjection chamber. A row consisting of two round droplets and three elongated drops was placed along the center line of the dish. The first droplet (3 μl) was formed from 10% polyvinylpyrrolidone (PVP) (Sigma) dissolved in Dulbecco phosphate-buffered saline (GIBCO BRL) and was for washing the pipette. The second droplet (3 μl) was a suspension of sperm in 10% PVP. The third elongated droplet (3 μl) was modified-HTF containing prepared sperm. The fourth and fifth elongated droplets (3 μl) were for the oocytes. These droplets were covered with mineral oil (Sigma). The dish was placed on the stage of an inverted microscope (Olympus, Tokyo, Japan) and its contents viewed with the aid of Nomarski differential interference optics. Motile sperm were aspirated from the suspension into the injection pipette, transferred to a drop containing an oocyte, and immobilized by crushing the tail between the injection pipette and the dish. Intracytoplasmic sperm injection was performed on the microscope. After injection, oocytes were transferred to growth medium consisting of HTF with 15% heat-inactivated maternal serum.