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Intracytoplasmic morphologically selected sperm injection is beneficial in cases of advanced maternal age: a prospective randomized study

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ABSTRACT

Objective: To evaluate advanced maternal age as a rationale for performing intracytoplasmic morphologically selected sperm injection (IMSI).
Study design: This study included couples undergoing intracytoplasmic sperm injection (ICSI) as a result of advanced maternal age (≥37 years old). Sample size calculations were based on the assumption that a 15% difference in implantation rate would mean a clinically significant difference. To achieve this difference, 33 cycles would be needed in each treatment arm (with a significance level of 5% and power of 85%). Couples were randomly allocated to one of two sperm selection procedures (ICSI, $n = 33$; or IMSI, $n = 33$). Sperm selection in the ICSI group was analyzed under a magnification of 400×. Sperm selection in the IMSI group was analyzed under high magnification of 6600×. The groups were compared with regard to the outcome of the cycles.
Results: IMSI cycles showed significantly higher implantation (4/33, 12.1% vs. 18/47, 38.3%, $p = 0.026$) and pregnancy (4/29, 13.8 vs. 18/30, 60.0%, $p < 0.001$) rates. The IMSI procedure positively influenced the blastocyst formation rate (RC: 15.00, R^2: 49.9%, $p = 0.001$) and implantation rate (RC: 24.04, R^2: 9.6, $p = 0.027$), and was determinant to the increased odds of pregnancy (OR: 9.0, CI: 2.17–37.38, $p = 0.001$).
Conclusion: It seems that the injection of a morphologically normal spermatozoon overcomes the low oocyte quality in older women, resulting in improved embryo quality and in a 9-fold increase in the clinical pregnancy rate in couples with advanced maternal age.

1. Introduction

A new concept of observation of sperm at high magnification in real time, known as motile sperm organelle morphology examination (MSOME), was recently introduced [1]. Its incorporation, together with a micromanipulation system, has allowed the introduction of a modified ICSI procedure, called 'intracytoplasmic morphologically selected sperm injection' (IMSI). Several publications reported that the IMSI procedure is positively associated with implantation and pregnancy rates [2–9]. Moreover, a recently published meta-analysis showed that the IMSI procedure is associated with improved embryo quality and improved implantation and pregnancy rates as well as lower miscarriage rates [10].

The high magnification allows the detection of sperm containing nuclear vacuoles. Previous studies demonstrated an association between sperm nuclear vacuoles and DNA decondensation [11], and DNA fragmentation [12,13].

It is known that human sperm have highly dynamic and essential participation in embryogenesis that clearly goes beyond the fertilization process. The first divisions of the newly formed embryo depend on the machinery of the oocyte. Activation of the embryonic genome occurs at the stage of 4–8 cells [14]. Studies suggest that this result reflects a late paternal effect, which is related to blockage of embryonic development observed during/after the implantation of embryos with normal karyotype [15,16].

The ability of the human oocyte to repair sperm DNA damage has not been fully elucidated, but some gene expression studies showed that the oocyte is equipped with mechanisms that can repair some of the paternal DNA abnormalities [17,18]. The ability to repair, however, depends on the type and extension of DNA damage, and mainly on the woman's age and oocyte quality [19,20].

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We therefore hypothesized that women with advanced maternal age could benefit from the injection of spermatozoa selected under high magnification. The aim of this study was to evaluate advanced maternal age as a rationale for performing IMSI.

2. Materials and methods

2.1. Experimental design, patients and inclusion criteria

This prospective randomized clinical trial was performed in a private fertility centre. Inclusion criteria were as follows: women of good physical and mental health, undergoing ICSI as a result of advanced maternal age (≥37 years old), with regular menstrual cycles of 25–35 days, normal basal FSH and LH levels, BMI less than 30 kg/m^2, presence of both ovaries and intact uterus, absence of polycystic ovaries, endometriosis, or gynaecological/medical disorders and a negative result in a screening for sexually transmitted diseases. All male partners were normozoospermic patients, according to the WHO reference values [21]. No patient had received any hormone therapy for at least 60 days preceding the study.

Written informed consent was obtained, in which patients agreed to share the outcomes of their own cycles for research purposes, and the study was approved by the local institutional review board.

2.2. Randomization protocol

Eligible patients who agreed to participate were randomly assigned to the ICSI group or IMSI group using a computer random number generator (Fig. 1). Randomization and the allocation sequence were provided by a computer generated random number list prepared by an investigator with no clinical involvement in the trial. The allocation sequence was concealed from the participant, the clinical care providers and the embryologists by retaining the entire sequence with the primary study team (principal investigator and study coordinator). Following patient consent for participation in the clinical trial, the embryologist received the allocation assignment. All participants and the clinical care providers were blinded to the allocated arm. Only the embryologists were aware of the group to which the participant was allocated.

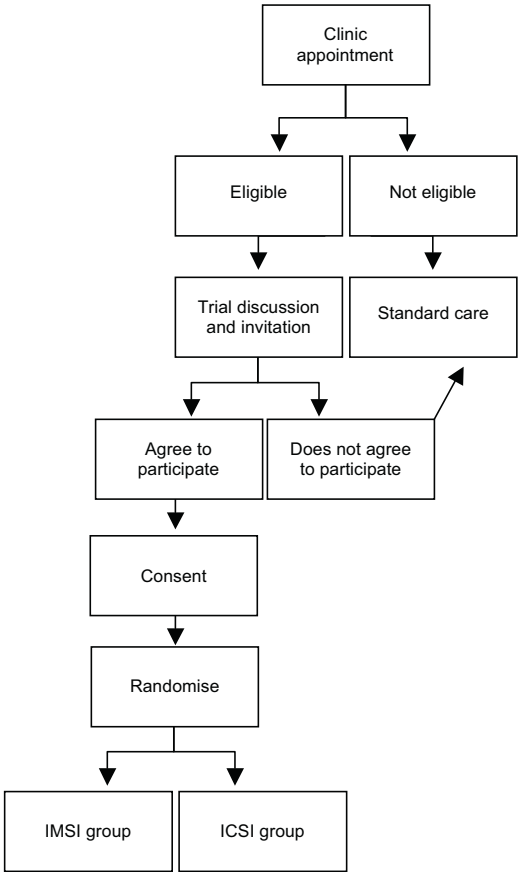

Fig. 1. Study design.

2.2.1. Controlled ovarian stimulation

Ovarian stimulation was achieved by the administration of a recombinant follicle-stimulating hormone (r-FSH, Gonal-F®, Serono, Geneve, Switzerland) on a daily basis until the visualization of at least one follicle ≥14 mm, at which time we began the administration of a gonadotropin-releasing hormone (GnRH) antagonist, cetrorelix acetate (Cetrotide; Serono Laboratories, Geneva, Switzerland) 0.25 mg subcutaneous (SC).

The ovulation trigger was given by SC injection of 250 μg of recombinant human chorionic gonadotrophin (hCG, Ovidrel™, Serono, Geneva, Switzerland) when at least three follicles ≥17 mm were observed. Oocyte retrieval was performed 35 h after the administration of hCG, through transvaginal ultrasonography.

2.2.2. Intracytoplasmic sperm injection procedures

2.2.2.1. ICSI. Sperm selection in the ICSI group was analyzed under a magnification of 400× using an inverted Nikon Eclipse TE 300 microscope. ICSI was performed in a micro-injection dish prepared with 4 μL droplets of buffered medium (Global® w/HEPES, LifeGlobal, Connecticut, USA) and covered with paraffin oil on a heated stage at 37.0 ± 0.5 °C of an inverted microscope.

2.2.2.2. IMSI. Sperm selection in the IMSI group was analyzed under high magnification using an inverted Nikon Diaphot microscope equipped with high-power differential interference contrast optics (DIC/Nomarski). The total calculated magnification was 6600×. An aliquot of the sperm cell suspension was transferred to a microdroplet of modified human tubal fluid medium containing 8% polyvinyl pyrrolidone (PVP; Irvine Scientific, Santa Ana, CA) in a sterile glass dish (FluoroDish; World Precision Instrument, Sarasota, FL). The dish was placed on a microscope stage above an Uplan Apo × 100 oil/1.35 objective lens previously covered by a droplet of immersion oil. The sperm cells exhibiting normally shaped nuclei (1) smooth, (2) symmetric, and (3) oval configuration) and (4) normal nuclear chromatin content (if it contained no more than one vacuole, which occupies <4% of the nuclear area) were selected for injection [1–3,17,18,22,23].

2.2.3. Fertilization, embryo quality and embryo transfer

Approximately 16 h after ICSI, fertilization was confirmed by the presence of two pronuclei and the extrusion of the second polar body. Embryos were maintained in a 50 μL drop of culture medium (Global®, LifeGlobal, Connecticut, USA) supplemented with 10% protein supplement covered with paraffin oil in a humidified atmosphere under 6% CO_2 at 37 °C for five days.

High-quality embryos were defined as those showing 8–10 cells on the third day of development, less than 15% fragmentation, symmetric blastomeres, absence of multinucleation and absence of zona pellucida dysmorphisms.

High-quality blastocysts were defined as full or expanded or hatching or hatched blastocysts, presenting a normal inner cell mass (ICM) and trophectoderm (TE). The ICM was classified as either high quality (tightly packed with many cells) or low quality (loosely grouped with several or few cells). Similarly, the TE was