ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Polarization microscopy imaging for the identification of unfertilized oocytes after short-term insemination

Yi Guo, M.D.,[a,b] Wenqiang Liu, Ph.D.,[a] Yu Wang, M.D.,[a] Jiaping Pan, M.D.,[a] Shanshan Liang, M.D.,[a] Jingling Ruan, M.D.,[a] and Xiaoming Teng, M.D.[a]

[a] Department of Assisted Reproduction, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, and [b] Department of Cellular and Genetic Medicine, Shanghai Medical College, Fudan University, Shanghai, People's Republic of China

Objective: To develop a unique approach using polarization microscopy (PM) to determine whether the presence of a spindle can be used as an indicator associated with fertilization failure 5 hours after short-term insemination.
Design: Observational study.
Setting: Assisted reproduction center.
Patient(s): Eighty-five patients undergoing short-term insemination.
Intervention(s): Oocytes imaged via PM at 4, 5, and 6 hours after standard insemination.
Main Outcome Measure(s): Spindle visualization and fertilization rate, with rescue intracytoplasmic sperm injection (ICSI) results determined by rates of normal fertilization, abnormal fertilization, and good-quality embryo formation.
Result(s): After standard insemination, comparisons of spindle visualization at three time points indicated that the predictive accuracy rates were 84.30% at 5 hours, 86.80% at 6 hours, and 62.20% at 4 hours, with the rates at 5 and 6 hours statistically significantly higher than at 4 hours. A spindle was present in 242 of the 788 metaphase-II oocytes 5 hours after insemination, and there were 204 failed fertilizations on day 1. The positive predictive value was 0.84. After rescue ICSI, the abnormal fertilization rate of the polar body group (assessed using the polar body visualization method) was statistically significantly higher than that of the PM group (assessed using the spindle visualization method) and the regular ICSI group (9.37%, 5.88%, and 4.87%, respectively).
Conclusion(s): The presence of a spindle 5 hours after insemination in in vitro fertilization is an accurate indicator of unfertilized oocytes. Spindle imaging combined with rescue measures effectively prevents fertilization failure and decreases the polyspermy rate. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Polarization microscopy, rescue ICSI, short-term insemination, spindle imaging, unfertilized oocyte

Fertilization failure occurring in standard in vitro fertilization (IVF) is one of the most frustrating experiences for both infertile couples and the reproductive team. Initially, late-rescue ICSI in day-1 unfertilized oocytes is often the first choice for total fertilization failure after conventional IVF (1, 2). Considering the poor developmental potential of the embryos and the low chance of pregnancy along with the increased genetic risks, late-rescue ICSI is not recommended (3, 4).

Short-term coincubation of gametes combined with early-rescue ICSI has been developed and is applied in many reproductive centers (5, 6). In traditional methods, second polar body (PB) extrusion is considered to be a reliable predictor for subsequent fertilization. However, confirming that oocytes without detectable second PBs 6 hours after insemination have not been penetrated by a spermatozoon or are not actually undergoing a delayed fertilization process remains difficult. Generating triploid embryos cannot be avoided if another spermatozoon is injected into the fertilized oocyte. In addition, the presence of fragmented PBs and pseudo double PBs makes identification more complicated, especially in cases of partial fertilization failure. Thus, early-rescue ICSI tends to lead to a higher rate of polyploidy (7).

Although this method has some drawbacks, early-rescue ICSI has resulted in better fertilization, pregnancy, and implantation rates compared with

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Y.G. and W.L. should be considered similar in author order.
Reprint requests: Xiaoming Teng, M.D., Department of Assisted Reproduction, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, 2699 Western Gaoke Road, Shanghai 200000, People's Republic of China (E-mail: tengxiaoming@hotmail.com).

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those of late-rescue ICSI (8). These results suggest that rescue ICSI will be more effective if oocytes can be rescued before they lose their developmental competence. If there is a method to identify an unfertilized oocyte shortly after standard insemination, early-rescue ICSI may salvage some oocytes' reproductive potential and the rate of induced polyspermy as a result of errors could be reduced, potentially increasing the number of available embryos. Meanwhile, the clinical evidence regarding rescue ICSI does not indicate an elevated rate of malformations although the data are limited and incomplete (9–11). It is considered an alternative method to prevent the occurrence of total fertilization failure or near total fertilization failure in IVF treatment.

Polarization microscopy (PM), which can provide a noninvasive means of identifying the meiotic spindle in human oocytes, has been used in assisted reproductive technology. Spindle imaging using this technique has been employed as a new marker for the optional timing of ICSI (12); it has also been useful for predicting in vitro–matured oocyte development and oocyte developmental potential after ICSI through the relationship between the position of the meiotic spindle and the PB (13, 14). Depending on whether the spindle is observed on day 1 after oocyte retrieval, unfertilized eggs rescued by late-ICSI have yielded varying results. Moon et al. (15) found that the normal fertilization rate in their study group with observation of the spindle was statistically significantly higher than in the group without observation of the spindle (68.7% vs. 43.7%) and that the rates of three- or four-pronuclei embryos were statistically significantly decreased in the study group compared with the rates in the group without observation of the spindle (4.5% vs. 26.4%).

In this study, we evaluated the value of spindle imaging in predicting unfertilized oocytes in short-term insemination. This approach was both objective and effective, particularly in cases of partial fertilization failure; we also shortened the time interval from insemination to rescue.

MATERIALS AND METHODS

Patients, Stimulation Protocol, and Oocyte Retrieval

This study was approved by the ethics committee of Shanghai First Maternity and Infant Hospital. Our retrospective analysis included a total of 85 patients who received short-term insemination between May 2015 and April 2016. The controlled ovarian hyperstimulation protocol and oocyte retrieval were performed as described by Yang et al. (16), and the details are briefly described as follows. The controlled ovarian hyperstimulation protocol consisted of gonadotropin-releasing hormone agonist down-regulation followed by follicle-stimulating hormone/human menopausal gonadotropins. Transvaginal ultrasound-guided oocyte retrieval was performed 36 hours after administration of 10,000 IU of human chorionic gonadotropin. Retrieved cumulus–corona oocyte complexes were washed with equilibrated G-MOPS (Vitrolife) and were then placed into Petri dishes containing G-IVF PLUS (Vitrolife) in a 5% O_2, 6% CO_2, 37°C incubator (Astec).

Sperm Preparation and Short-term Insemination

Semen samples were collected by masturbation after 3 to 5 days of sexual abstinence on the day of oocyte retrieval. Semen analysis was performed according to the World Health Organization manual (17). The sperm swim-up method was conducted as follows: after 30 minutes of liquefaction in a 37°C incubator, 3 mL of culture medium (G-IVF PLUS) was gently stratified above the semen. The tube was inclined at a 45° angle and was incubated for 1 hour (37°C, 6% CO_2). The supernatant was then transferred into an empty tube and centrifuged for 5 minutes at 300 × *g*. The sperm pellet was resuspended with warmed G-IVF Plus medium and maintained in a 6% CO, 37°C incubator (Thermo Scientific) until insemination.

Three to four cumulus oocyte complexes were placed into 100 μL of G-IVF PLUS droplets covered by mineral oil (Vitrolife), and each oocyte was inseminated with 30,000 to 40,000 motile spermatozoa in a 5% O_2, 6% CO_2, 37°C incubator. Four hours after insemination, the cumulus cells were mechanically removed.

Prediction of Unfertilized Oocytes and Assessment of Fertilization

This study was divided into two groups according to different identification methods. In the group using polarization microscopy for visualization (PM group), each oocyte was placed into individual 15-μL droplets of G-1 Plus (Vitrolife) covered with mineral oil in a glass-bottom culture dish (Fluorodish; World Precision Instruments). The oocytes were determined to be unfertilized (oocyte failed activation) when a spindle was observed under PM (Oosight; Hamilton Thorne), regardless of whether one, two, or fragmented polar bodies were present (Fig. 1A–F).

With the PB visualization method (PB group), oocytes were determined to be unfertilized (oocyte failed activation) when only one PB was observed under the inverted microscope (see Fig. 1G). The appearance of 2PBs (double polar bodies) indicated that fertilization was occurring (oocyte activated) (see Fig. 1H). The end result of fertilization was determined by checking for the presence of ≥1 pronucleus on day 1 (18–22 hours after insemination). Eggs without any pronucleus were considered to be unfertilized. Embryonic development was assessed on day 2 (48 hours) and day 3 (72 hours) according to the number and regularity of blastomeres and the percentage and pattern of anucleate fragments. In this study, we defined embryos as good quality embryos on day 3 if they had between seven and nine blastomeres and <15% anucleate fragments and no apparent morphologic abnormalities. Embryos showing blastomere multinucleation, uneven cell division, and cytoplasmic abnormalities were defined as low quality. All the observation processes above were performed in a 37°C constant temperature device (IXCBIB200; Olympus), which consists of an electric heater and an enclosed box around the microscope table.

Rescue ICSI Procedure

Five hours after insemination, the oocytes considered by spindle visualization to be unfertilized were microinjected with