The impact of examining the meiotic spindle by polarization microscopy on assisted reproduction outcomes

Maria C. Picinato, B.Sc.,[a] Wellington P. Martins, Ph.D.,[a,b,c] Roberta C. Giorgenon, B.Sc.,[a] Camila K. B. Santos, Ph.D.,[a] Rui A. Ferriani, Ph.D.,[a,b] Paula A. A. S. Navarro, Ph.D.,[a,b] and Ana C. J. de Sá Rosa-e-Silva, Ph.D.[a,b]

[a] Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirão Preto, University of São Paulo; [b] National Institute of Science and Technology, National Counsel of Technological and Scientific Development, Hormones and Women's Health; and [c] School of Ultrasonography and Medical Recycling of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil

Objective: To examine the effect of submitting oocytes to polarization microscopy (PM) before intracytoplasmic sperm injection (ICSI).
Design: Retrospective observational study.
Setting: University hospital in Brazil.
Patient(s): Couples undergoing ICSI.
Intervention(s): PM before ICSI (PM group) compared with no PM before ICSI (No-PM group)
Main Outcomes Measure(s): Fertilization and cleavage rates, formation of top-quality embryos (TQEs), and implantation, clinical pregnancy, miscarriage, and live-birth rates.
Result(s): The PM group consisted of 1,000 consecutive oocytes from 201 couples submitted to PM during the year of 2008. The No-PM group consisted of 1,400 oocytes from 249 couples: 700 consecutive oocytes were retrieved before we started using PM and 700 consecutive oocytes were retrieved after we stopped using PM. In the PM group, we observed an increased fertilization rate (79.7% vs. 72.5%, PM group vs. No-PM group, respectively) but reduced cleavage rate (86.2% vs. 92.5%) and TQE formation (33.1% vs. 49.9%). Implantation (18.7% vs. 20.6%), clinical pregnancy (31.8% vs. 33.3%), miscarriage (21.9% vs. 15.7%), and live-birth (24.9% vs. 28.1%) rates were not significantly different between groups.
Conclusion(s): Use of PM was associated with increased fertilization rate but reduced cleavage rate and TQE formation; no significant difference was observed for implantation, clinical pregnancy, or live-birth rates. (Fertil Steril® 2014;101:379–84. ©2014 by American Society for Reproductive Medicine.)
Key Words: Oocytes, polarization microscopy, assisted reproductive techniques, comparative study

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/picinatomc-polarization-microscopy-assisted-reproductive-technology/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

The meiotic spindle (MS) of human oocytes in metaphase II (MII) is a temporary dynamic structure consisting of microtubules that is associated with the oocyte cortex and its network of subcortical microfilaments (1–3). The MS microtubules are linked to the kinetochores of the chromosomes (4) and participate in segregation during meiosis. It is known that the oocyte MS integrity is important for chromosome segregation (5–7) and that it is extremely sensitive to various factors such as aging, thermal changes, insufficient oxygen supply during culture, and oocyte manipulation (8–10). Damage to the MS may contribute to aneuploidy during the second meiotic division after fertilization and to the development of embryos of poor quality, especially in women older than 40 (7, 11).

Received June 12, 2013; revised September 20, 2013; accepted October 7, 2013; published online November 9, 2013.
M.C.P. has nothing to disclose. W.P.M. reports personal fees from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado de São Paulo, and Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo; and has received grants from Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas a Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo. R.C.G. has nothing to disclose. C.K.B.S. has nothing to disclose. R.A.F. has nothing to disclose. P.A.A.S.N. has nothing to disclose. A.C.J.d.S. has nothing to disclose.
Reprint requests: Ana C. J. de Sá Rosa-e-Silva, Ph.D., Setor de Reprodução Humana, Departamento de Ginecologia e Obstetrícia da Faculdade de Medicina de Ribeirão Preto, University of São Paulo, Avenida Bandeirantes, 3900-8, andar Monte Alegre, 14049-900, Ribeirão Preto, São Paulo, Brasil (E-mail: anasars@fmrp.usp.br).

Fertility and Sterility® Vol. 101, No. 2, February 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.10.009

Several studies have shown a correlation between the presence of MS identified by polarization microscopy (PM) and an increased fertilization rate and embryo quality (12–18) in women undergoing assisted reproductive techniques (ART). Additionally, the identification of the location of the MS in the oocyte might prevent damage to this structure during intracytoplasmic sperm injection (ICSI) (19, 20). However, some studies have questioned the usefulness of MS evaluation by PM since it did not improve fertilization or early embryo quality (13, 21) and because there is a potential deleterious impact on embryo development since additional oocyte handling is necessary.

The objective of the present study was to compare the reproductive outcomes of women undergoing ART during a period when PM was routinely performed before ICSI with those observed when PM was not used.

MATERIALS AND METHODS

Study Design and Context

This was a retrospective observational study evaluating the data from women who were submitted to ART in our assisted reproduction center. The study was approved by our local Ethics Committee. No informed consent was asked for the women owing to the nature of this study (retrospective analysis).

Participants

In our assisted reproduction center, all oocytes with first polar body (PB) extrusion determined by light microscopy were routinely subjected to PM before ICSI during the year 2008: we planned to compare the results observed by 1,000 consecutive oocytes with first PB extrusion determined by light microscopy submitted to PM during this year (PM group) with those observed in 1,400 consecutive oocytes with first PB extrusion determined by light microscopy that were retrieved but that were not submitted to PM: 700 consecutive oocytes retrieved in the period before we started using PM and more 700 retrieved after we stopped using PM (No-PM group).

Variables

Fertilization rate: the number of oocytes that presented two distinct pronuclei and two PBs 18–20 hours after ICSI divided by the number of injected oocytes.

Cleavage rate: the number of cleaved embryos 40–44 hours after ICSI divided by the number of fertilized oocytes.

Top-quality embryo (TQE) formation: the number of 4-cell embryos with symmetrical blastomeres and no fragmentation 40–44 hours after ICSI divided by the number of fertilized oocytes.

Implantation rate: the number of gestational sacs observed divided by the number of embryos transferred.

Positive pregnancy test rate: the number of women who had a positive pregnancy test divided by the number of women who had at least one MII oocyte.

Clinical pregnancy rate: the number of women who had at least one gestational sac observed by ultrasound divided by the number of women who had at least one MII oocyte.

Live-birth rate: the number of women who delivered at least one living baby divided by the number of women who had at least one MII oocyte.

Miscarriage rate: the number of women who had a miscarriage (including ectopic pregnancy) divided by the number of women who had a clinical pregnancy.

We also analyzed the causes of infertility as the proportion of included couples who had the following diagnoses: endometriosis, anovulation, tubal, and male factor. These causes were not mutually exclusive, for example, one couple could have more than one cause.

Controlled Ovarian Stimulation (COS), Oocyte Retrieval, and Gamete Preparation

All women were subjected to COS before oocyte retrieval according to the standard long protocol, using gonadotropins (150–300 IU/day) and GnRH analogues. Recombinant hCG was administered when two or more follicles reached a mean diameter $\geq$18 mm, and oocytes were retrieved 34–36 hours after.

The retrieved oocytes were incubated in 5% CO_2 at 37°C and 95% humidity for 2 hours and then denuded and placed in 5-μL drops on the lower portion of a glass Petri dish (Willco-dish; Willco Wells). Approximately 2 hours after retrieval, the oocytes were denuded, placed individually in a 5-μL drop of HTF-Hepes with 10% synthetic serum substitute, and evaluated for the presence of the first PB to classify their maturity.

Semen samples for the procedure were collected solely by masturbation. Fresh semen samples were processed by the discontinuous gradient technique (90%–45%) and centrifuged at 1,000 rpm for 30 minutes (22).

PM

In the No-PM group, ICSI was performed immediately after denudation.

In the PM group, the oocytes classified as mature were immediately analyzed by PM for the presence or absence of the MS and its location in relation to the PB. The OCTAX ICSI Guard System (Medical Technology Vertriebs-GmbH) controlled by a computer using the OCTAX EyeWare software (Universal Imaging Corp.), was used to visualize the MS and to capture the image immediately before ICSI. All readings were performed by the same observer (M.C.P.), a biologist with 20 years of experience in ART. This observer spent approximately 30–60 seconds to perform PM. ICSI was performed just after PM.

The oocytes were classified by PM into three groups:

1. Unidentifiable MS.
2. Identifiable MS not completely located inside the oocyte cytoplasm: telophase I (TI).
3. Identifiable MS completely located inside the oocyte cytoplasm: MII.

Additionally, the oocytes with identifiable MS in MII were subdivided into six groups according to the angle detected between the MS and PB: position 1 = 0°–30°, position