ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Cumulus oophorus complexes favor physiologic selection of spermatozoa for intracytoplasmic sperm injection

Caizhu Wang, M.S., Guixue Feng, Ph.D., Jinhui Shu, M.S., Hong Zhou, M.S., Bo Zhang, B.S., Huanhua Chen, M.S., Ruoyun Lin, M.S., Xianyou Gan, M.S., Zhulian Wu, M.S., and Tinglv Wei, B.S.

Center of Reproductive Medicine, Guangxi Maternal and Child Health Hospital, Nanning, Guangxi, People's Republic of China

Objective: To investigate the effectiveness of cumulus oophorus complexes (COCs) in the physiologic selection of spermatozoa for intracytoplasmic sperm injection (ICSI).
Design: A prospective sibling oocytes study.
Setting: Center of reproductive medicine.
Patient(s): Couples undergoing ICSI during 2016, females aged ≤38 years, and at least six metaphase II (MII) oocytes retrieved. Sixty patients were included in the study. Of 857 MII oocytes, 429 were allocated to the study group and were injected with the sperm selected via COCs; 428 MII oocytes were allocated as controls (C) and fertilized by conventional ICSI.
Intervention(s): In the study group, ICSI was performed with spermatozoa that traversed the COCs in vitro.
Main Outcomes Measure(s): Blastocyst/top blastocyst formation rate, fertilization rate, and oocyte utilization rate.
Result(s): Oocytes injected with COC-selected spermatozoa had a significantly higher fertilization rate than the conventional ICSI group (85.31% vs. 74.77%). There were no statistically differences in cleavage and top embryo rate on day 3 between the COC-ICSI and C-ICSI groups. However, with day 5 or 6 embryos, compared with conventional ICSI, COC-ICSI significantly improved blastocyst formation rate (64.90% vs. 53.50%), blastocyst formation rate at day 5 (46.52% vs. 38.85%), top blastocyst rate (38.72% vs. 24.20%), and the usable blastocysts formation rate (62.12% vs. 46.82%). The oocyte utilization rate was improved greatly in the COC-ICSI group compared with the C-ICSI group (51.98% vs. 34.35%). Furthermore, the fertilization rate, top embryo rate on day 3, usable blastocyst rate, top blastocyst rate, and day 5 usable blastocysts rate were similar between the conventional IVF and COC-ICSI groups. Single-blastocyst transfer was performed in 82 cycles, including 44 fresh cycles and 38 frozen-thawed cycles. The cumulative embryo implantation rate in the COC-ICSI group was 64.29%, slightly higher than in the C-ICSI group (53.85%), but there was no statistical difference.
Conclusion(s): The use of COCs to select spermatozoa for ICSI appears to be effective and led to a statistically significant improvement in blastocyst development and quality. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Intracytoplasmic sperm injection, cumulus oophorus complexes, sperm selection, sibling oocytes, blastocyst

Discuss: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/28830-25129

Nowadays, intracytoplasmic sperm injection (ICSI) is commonly used as a technique to overcome severe male-factor infertility, and it has been used mostly for the following indications: severe oligospermia, asthenospermia, teratospermia, and low fertilization or failed fertilization in previous standard in vitro fertilization (IVF) cycles. However, in contrast to conventional IVF, ICSI bypasses all natural barriers that can prevent the entry of a "bad" spermatozoon into an oocyte, thereby affecting embryo development and increasing the risk of genetic defects (1, 2). Therefore, choosing a healthy mature sperm for fertilization before ICSI is especially critical. It is well known that poor semen quality is related to impaired sperm DNA integrity and that sperm DNA damage has a negative effect on embryo development and clinical pregnancy (3–8). Miller et al. demonstrated that performing ICSI in cases of severe male-factor infertility may have a detrimental effect on blastocyst development and quality (9). Furthermore, Bodri et al. found, by

Received October 9, 2017; revised December 19, 2017; accepted December 20, 2017.
C.W. has nothing to disclose. G.F. has nothing to disclose. J.H. has nothing to disclose. H.Z. has nothing to disclose. B.Z. has nothing to disclose. H.C. has nothing to disclose. R.L. has nothing to disclose. X.G. has nothing to disclose. Z.W. has nothing to disclose. T.W. has nothing to disclose.
C.W and G.F should be considered similar in author order.
Supported by a project grant from the Research Foundation of Guangxi Medical and Health (project no. S201612) and a project grant from the Self-Raised Foundation of Guangxi Health Commission (project no. Z2016093).
Reprint requests: Caizhu Wang, M.S., Center of Reproductive Medicine, Guangxi Maternal and Child Health Hospital, Nanning, Guangxi 530003, People's Republic of China (E-mail: 277968256@qq.com).

Fertility and Sterility® Vol. ■, No. ■, ■ 2018 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2017.12.026

monitoring 500 blastocyst using time-lapse photography, that at the expanded blastocyst stage IVF-fertilized embryos showed faster development than ICSI-fertilized embryos (10). Therefore, a technique to select an ideal sperm for use in ICSI could improve the embryo development potential and optimize the outcome of the treatment (11–13).

In general, spermatozoa are visually selected for ICSI under ×400 optical magnification on the basis of their morphology and motility. However, this approach does not reflect the genomic integrity of the spermatozoon and its ability to provide the best paternal contribution to the zygote.

The cumulus oophorus complex (COC) surrounds the oocyte and consists of the cumulus cells and their extracellular matrix (ECM). During natural fertilization, only the spermatozoa that traverse the COC get the chance to reach and penetrate the zona pellucida and fertilize the oocyte. If an ovulated oocyte is completely devoid of the COC, it remains unfertilized (14). The ECM is a complex structure, the major component of which is hyaluronic acid (HA), which is synthesized by the cumulus cells after the LH surge (15) and is considered to play a pivotal role in the selection of healthy spermatozoa (16, 17).

Previous studies have shown that spermatozoa that pass through the COC have better sperm morphology and are more acrosome reacted. In addition, the spermatozoa have a higher zona-binding capacity and higher chromatin integrity (18–20). Therefore, it seems that COCs can also play a role in sperm selection. We refer to this novel sperm selection method as physiologic ICSI. However, to date, there are no data regarding this selection method on the development of the embryo. The aim of the present study was to evaluate the effectiveness of COCs in the physiologic selection of spermatozoa for ICSI therapy.

MATERIALS AND METHODS

Subjects

Sixty couples who underwent ICSI cycles from January to December 2016 were included prospectively in the study. The inclusion criteria were as follows: ICSI indications in our clinic; women aged ≤38 years; and at least six metaphase II (MII) oocytes retrieved. The first exclusion criterion was to exclude cycles with thawed semen or semen from testicular aspiration. The second exclusion criterion was to exclude patients whose spermatozoa did not pass through the COCs after 1 hour of the prepared spermatozoa being added into the COC selection model. The third exclusion criterion was to exclude the patients who did not choose to perform blastocyst culture on day 3.

Ovarian Stimulation

Stimulation protocols were performed according to the routine scheme developed by our clinic. Briefly, all patients were down-regulated with the use of leuprolide acetate (Lupron; TAP Pharmaceuticals, Lake Forest, Illinois). Ovarian stimulation was achieved with the use of recombinant FSH (Gonal-F or Puregon; Merck Serono, Italy). When two or more follicles reached ≥18 mm in mean diameter, 5,000–10,000 IU hCG (Serono, Switzerland; or Livzon, China) was administered. Oocytes were collected by means of follicular aspiration with the use of vaginal ultrasonography 34–36 hours after hCG administration.

Collection of COCs

While cumulus-oocyte complexes were picked up, the excess COCs were partially dissected mechanically with the use of a glass pipette under an optical microscope. Then the obtained COCs were collected, pooled in culture medium (Quinn Advantage medium, ART-1026; Sage) supplemented with human serum albumin (HAS) (Quinn Advantage SPS serum protein substitute, ART-3010; Sage), and incubated at 37°C in 5% CO_2 while awaiting further use. Meanwhile, the cumulus-oocyte complexes were also transferred to the incubator quickly and incubated at 37°C in 5% CO_2 in culture media (Quinn Advantage medium, ART-1026) supplemented with HAS (Quinn Advantage SPS serum protein substitute, ART-3010).

Spermatozoa Preparation

Semen samples were collected via masturbation after 3–5 days of ejaculatory abstinence or by percutaneous epididymal sperm aspiration. Semen samples with low or failed fertilization capability in previous IVF cycles were processed with the use of direct swim-up. Briefly, 1 mL medium (Quinn Advantage medium, ART-1020; Sage) supplemented with HAS (Quinn advantage SPS serum protein substitute, ART-3010; Sage) was placed in a sterile 15-mL centrifuge tube, and 1 mL liquefied semen was carefully layered under the medium. This was then incubated for 1 hour at 37°C, and the uppermost 0.5 mL of medium was collected for further use. Other semen samples, including ejaculation and percutaneous epididymal sperm aspirations, were prepared by simple washing. Briefly, semen samples were diluted with the use of 3 mL medium and centrifuged for 5 minutes at 500*g* at room temperature. After supernate removal, the pellet was collected for further use.

Oocyte Preparation and Randomization

After incubation for 3 h, the cumulus cells were denuded from cumulus-oocyte complexes with the use of hyaluronidase solution by means of a Pasteur pipette before ICSI. After final denudation, only the oocytes at MII stage were identified. Sibling MII oocytes were equally allocated to two separate culture dishes in a randomized way. A computer-generated randomization list was used for allocation of the first dish to either study group (ICSI after sperm selection by COCs, named the COC-ICSI group) or the control group (conventional ICSI, named C-ICSI).

Spermatozoa Selection by COCs

In each experiment, we used the patient's own COCs in all cases. One hour before the ICSI procedure, the collected COCs were placed in an area, designated B1, of the ICSI operation dish in the middle of a channel with sperm wash