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Study Protocol&
Statistical Analysis

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Objective:

A total of 335 couples participated in this study, which was performed between January 2018 and December 2019 at the Reproductive Medicine Department, Jinan Central Hospital Affiliated to Shandong University, and Reproductive Medicine Center, Gansu Provincial Maternity and Child-care Hospital. The couples underwent ICSI/IVF treatment and their high quality embryos were randomized to embryo transfer using either fresh or self-spent medium with informed consents and ethics approvals.

Design:

Patients met the following criteria were included: (i) $\leq$ 35 years old; (ii) regular menstrual cycles; (iii) baseline follicle stimulating hormone (FSH) <12 IU/L; (iv)infertility with simple tubal or/and male factors; (v) did not experience more than two prior failed ET cycles; (vi) at least two good-quality Day 3 embryo or one good-quality Day 5 blastocyst for ET. Exclusion criteria were as follows: (i) uterine abnormalities, such as adenomyosis, endometriosis and endometrial polyps; (ii) ovarian abnormalities, such as low ovarian response and polycystic ovary syndrome (PCOS); (iii) endometrial thickness <7 mm on transfer day.

Methods:

Ovarian stimulation and IVF/ICSI procedure

Controlled ovarian hyperstimulation was carried out with uninterrupted injection of recombinant FSH (Gonal-F; Merck Serono; Switzerland) following pituitary down-regulation by GnRHa. When the dominant follicles measured $\geq$ 18 mm in diameter, human chorionic gonadotrophin (hCG, Lizhu Inc, China) or recombinant hCG (Ovidrel, Merck Sereno, Switzerland) was administrated, and oocyte retrieval was performed approximately 36.5 h later. Sperms were prepared using density gradient centrifugation and swim-up techniques before fertilization, and IVF/ICSI procedures were applied 4–6 h after oocyte retrieval. Fertilization was assessed by checking the appearance of two pronuclei 16–18 h later.

Embryo culture

After fertilization, embryos were cultured one by one in G1^{TM}-PLUS medium drops (Vitrolife, V.Frölunda, Sweden) containing human serum albumin and gentamicin covered with OVOIL^{TM} (Vitrolife, V.Frölunda, Sweden) in the culture dish until Day 3, G2^{TM}-PLUS medium was sequential used from Day 3 to Day 6 if the embryos need to blastocyst culture. All mediums for embryo culture were pre-equilibrated at 37°C and 6% CO_2 before use.

Cleavage stage embryos were morphologically classified according to the number and shape of blastomeres, the percentage of fragmentation as well as multinucleation on Day 3, as following: Grade 1, no or <5% fragmentation with at least six equal-sized blastomeres and no multinucleation; Grade 2, <20% fragmentation with slightly unequal-sized blastomeres and no evidence of multinucleation; Grade 3, 20%–50% fragmentation with obviously unequal-sized cells; and Grade 4, >50% damage or severely unequal-sized cells or visible multinucleation. Blastocysts were classified according to development stage (staging 1-5) and morphology of inner cell mass (Grading A-C) and trophectoderm (Grading A-C).

Embryo transfer

Two Day 3 fresh cleavage stage embryos scored better than grade 2 or single Day 5 fresh blastocyst stage embryo scored better than 4BB was selected for intrauterine transfer. The selected embryos were pre-transferred into a 500 μl pre-equilibrated fresh culture medium (G1^{TM}-PLUS or G2^{TM}-PLUS) awaiting ET. In the test group, the stretched thin Pasteur pipettes were used to absorb the self-spent droplets under the oil, and the droplets were transferred at least three times in a new dish to remove the oil floating on the surface. Then, the selected embryos were transferred to the combined droplet for catheter loading. Correspondingly, the embryos of the control group were loaded directly in the pre-equilibrated fresh culture medium. Luteal support by progesterone administration was provided to the patients after transfer.

Outcome Measures

Chemical pregnancy was confirmed by detecting serum β-hCG level 14 days after embryo transfer, and clinical pregnancy was defined as presence of at least one gestational sac identified by ultrasound 5 weeks after transfer. Biochemical pregnancy rate was defined as the number of early pregnancy losses divided by the total number of positive serum β-hCG. Clinical pregnancy rate was defined as the number of clinical pregnancies divided by the number of transfer cycles. Implantation rate was calculated as the number of geastational sacs divided by the number of embryos transferred. Clinical abortion rate was defined as the number of clinical pregnancy losses before 28 weeks of gestation divided by the total number of clinical pregnancies. Live birth rate was defined as a ratio between healthy living newborns and embryo transfer cycles.

Statistical Analysis:

Data were analyzed using student's t-test and chi-square test. $P<.05$ was considered statistically significant.