RESEARCH PROPOSAL

Version 1

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1. General Information

Title:

A randomized double blinded study comparing the live birth rate of IVF treatment following brief incubation of oocytes and spermatozoa versus standard incubation

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2. Background

In vitro fertilization (IVF) treatment is now commonly used to treat infertile couples. During IVF treatment, oocytes and sperm are routinely incubated overnight and this may lead to suboptimal culture conditions because of increased reactive oxygen species (ROS) produced by sperm in the standard incubation [1]. High levels of ROS can adversely affect the quality of the embryos, result in hardening of the zona pellucida and impair the implantation capacity of embryos [2-4]. Studies shows successful fertilization of an oocyte occurs 20mins after the gametes are put together [5]. Sperm can penetrate through the cumulus cells within 15min, and 80% of oocytes can be fertilized when they are exposed to a large number of spermatozoa within 1hr

[1]. As an attempt to avoid possible detrimental effects on the oocytes from long exposure to sperm, the brief incubation insemination protocol was developed. It implies that prolonged incubation of oocytes and sperm may not be necessary and may even be harmful. Some reports suggest that a sperm-oocyte exposure time of 1−6 h improves IVF outcomes [1,2,6,7] However, other studies report no such advantage with a short insemination time [8,9,10] .

A recent meta-analysis shows [11] brief incubation of gametes was associated with significantly higher rates of clinical pregnancy (RR: 1.84, 95% CI: 1.24−2.73), ongoing pregnancy (RR: 1.73, 95%CI: 1.27−2.33) and higher rate of implantation (RR: 1.80, 95% CI: 1.43−2.26) than standard incubation. But the rates of normal fertilization (RR: 0.98, 95% CI: 0.93−1.02), good quality embryos (RR: 1.24, 95% CI: 1.0−1.53) and polyspermy (RR: 0.84, 95% CI: 0.7−1.01) were not significantly different compared with standard incubation.

In a Cochrane meta-analysis [12] eight RCTs with 733 women were included, and showed similar results. But it only reported clinical pregnancy rate and ongoing pregnancy rate which were significantly higher in brief incubation group than standard incubation. For the miscarriage rate, there were six miscarriages in one trial including 167 women. This low quality evidence suggested no significant difference in the odds of miscarriage between brief incubation and standard incubation (OR 1.98, 95% CI 0.35 to 11.09; P = 0.44). It is important to note that the live birth rate, which is the important outcome parameter, was not reported in all these studies. It is uncertain whether brief incubation improves the life birth rate compared with standard incubation.

Drawbacks inherent to the quality of these studies, such as lack of allocation concealment and no blinding, limit the quality of the available evidence.

3. Objectives:

The aim of this randomized double blinded study is to compare the live birth rate of IVF treatment following brief incubation of oocytes and sperm versus standard incubation. The hypothesis is that a brief incubation improves the live birth rate of IVF t reatment.

4. Trial design:

Eligible patients will be recruited and randomized into one of the following two groups by computer-generated random numbers that will be placed in sealed envelopes:

1. Brief incubation group: oocytes will be exposed to spermatozoa for 2 hours
2. Standard incubation group: oocytes will be exposed to spermatozoa for 20 hours

5. Subject selection and exclusion

The target population for the trial will be women undergoing IVF treatment in our unit

5.1 Inclusion criteria:

- ❑ Women under 42 years of age
- ❑ Infertile for >=1 year
- ❑ Indications for conventional insemination such as tubal, endometriosis, unexplained and mild male factor
- ❑ Written informed consent

5.2 Exclusion criteria:

- ❑ Women using donor eggs/donor sperm
- ❑ Women with abnormal uterine cavity shown on hysterosalpingogram or saline infusion sonogram
- ❑ Women with hydrosalpinges shown on scanning and not treated
- ❑ Husband with severe oligospermia or azospermia or teraspermia (nomal morphology<5%)
- ❑ Previous fertilization failure for conventional insemination in the past (fertilization rate<30%)

6. Treatment of subjects

All techniques of IVF including ovarian stimulation will be provided according to local protocols.

6.1 Ovarian stimulation and IVF

All women will start their IVF treatment with ovarian stimulation using either the long agonist or antagonist protocols. For long protocol, gonadotropin-releasing hormone analogue (GnRHa) will be given for pituitary desensitization, on Day 2−3 of the menstrual cycle, they will undergo transvaginal ultrasound examination and serum E2 measurement. Human

menopausal gonadotrophin (hMG) or recombinant FSH will be given at 150−300 IU per day based on the antral follicle count, age of women and previous ovarian response, according to the standard operation procedures of the centre. Ovarian response will be monitored by serial transvaginal scanning with or without hormonal monitoring. Further dosage adjustments will base on the ovarian response at the discretion of the clinicians in charge. For antagonist protocols, antagonist (cetritide0.25mg daily) will be given on the 6th day of ovarian stimulation until the day of hcg trigger.

When three leading follicles reach ≥18 mm, hCG 10 000 IU or ovidrel 250 mg will be given to trigger final maturation of oocytes. Oocyte retrieval will be arranged around 36 h later.

6.2 Insemination Procedures

All semen samples were prepared by the conventional swim-up procedure. At 2hr after oocyte retrieval, each matured oocyte will be inseminated with approximately 50,000–100,000 motile spermatozoa.

6.3 Assignment and masking

On the day of OPU, patients will be randomized after occyte pick up by a laboratory technician according to a computer generated randomization list in sealed envelopes into the brief incubation group or the standard incubation group. Until the completion of the whole study, both the patients and the clinicians were blinded to the group assigned.

Control arm

Women who allocated to the standard incubation group, all oocytes will be exposed to spermatozoa for 20 hours, and checked for fertilization on day 1 (20 hours) after denuding

Intervention arm

For those allocated to the brief incubation group, all oocytes will be exposed to spermatozoa for 2 hours and gently washed through two organ dishes containing 1.5 ml of equilibrated medium, and then transferred to the corresponding microdroplet of equilibrated fresh medium. Surrounding cumulus cell will be retained, not removed from the ooctyes.

6.4 Fertilization and Embryo Evaluation

Both groups of oocytes will be decoronated and checked for the presence of two pronuclei and two polar bodies to confirm fertilization. All other

outcomes (i.e., no fertilization, one pronucleus, polyspermia, degeneration) will be recorded.

Embryo morphology will be assessed every day of culture. Embryos will be scored according to the following criteria. Briefly, embryos are classified as: gradeA, no cellular fragmentation; grade B, <20% fragmentation; grade C, 20% to 50% fragmentation; and grade D, >50% fragmentation. The number of blastomeres per embryo was also recorded. Grade A and B embryos constituted the “good quality embryo”.

The transfer is performed by the team clinician with a maximum of 2 embryos being replaced according to the standard protocol under transabdominal ultrasound guidance. Luteal phase support is given at the discretion of the physician.

6.5 Follow up strategy:

A pregnancy test will be carried out 2 weeks after embryo transfer in both arms. All women who have a positive pregnancy test 2 weeks after a fresh embryo transfer will undergo a transvaginal ultrasound scan after a further 2 weeks to identify the presence of a gestation sac with a fetal heart signifying an ongoing pregnancy.

Data on all pregnancy outcomes including early pregnancy losses such as miscarriage or ectopic pregnancy will be collected.

In order to achieve consistency with respect to the collection of outcome, standardised case report forms (CRF) will be completed for each woman at each centre. These CRFs will include details on treatment received, pregnancy outcomes, complications in pregnancy, mode of delivery and birth outcomes.

7. Study outcomes

Primary outcome is the live birth rate.

Secondary outcomes:

- Miscarriage rate
- Clinical pregnancy rates
- Normal fertilization rate
- polyspermy rate(>=3PN)
- Cleavage rate
- High quality embryo formation rate
- Implantation rate

8. Statistics

8.1 Analysis plan:

Demographic factors and clinical characteristics collected as part of baseline data collection will be summarised with counts (percentages) for categorical variables, mean (standard deviation [SD]) for normally distributed continuous variables, or median (interquartile [IQR] or entire range) for other continuous variables.

Outcomes for participants will be analysed in the groups to which they are assigned regardless of deviation from the protocol or treatment received (intention-to-treat). Comparative statistical analysis will entail calculating the relative risk (RR) (95% CI) for the primary outcome (99% CIs for all dichotomous secondary outcomes), the mean difference (99% CI) for normally distributed continuous secondary outcomes, or the median difference (99% CI) for skewed continuous variables.

All analyses will adjust for the minimisation factors to account for the correlation between treatment groups introduced by balancing the randomisation (which forces outcomes between treatment arms to be similar apart from any treatment effect).

Adjusted risk ratios will be estimated using a log binomial regression model, or using a log Poisson regression model with a robust variance estimator if the binomial model fails to converge. Linear regression will be used for normally distributed outcomes and quantile regression for skewed continuous variables.

Pre-specified subgroup analyses will include (i) Natural versus hormone replacement frozen replacement cycles, and (ii) Cleavage versus blastocyst stage transfer.

9. Sample size estimation:

The average live birth rate in our center from 2010-2012 was 30% per transfer. According to the recent meta-analysis[11], briefincubation of gametes was associated with significantly higher rates of ongoing pregnancy (RR: 1.73,

95%CI: 1.27−2.33) than standard insemination.

Assuming live birth in the brief incubation group increase from 30% to 50% (increase by 70%); with 90% power and a two-sided 5% level of statistical significance, we will need to recruit 124 women in each arm. To account for 10% loss to follow up, we will recruit 140 subjects in each group or 280 subjects for the whole study.

10. Assessment of safety

Results from a recent meta-analysis [11,12] showed the rates of normal fertilization, good quality embryos and polyspermy were not significantly different with short time incubation of gametes compared with standard insemination. Thus, there should be no major safety concern in this study.

11. Direct access to source data / documents:

Trial-related monitoring, audits, IRB review and regulatory inspections are allowed.

12. Quality control and quality assurance

Patients will be managed by the listed investigators who have adequate experience in managing assisted reproduction treatments. A Trial steering committee will be formed which will oversee the conduct of the trial.

13. Ethics

Written consent will be obtained from all patients. The patient information sheet and consent forms in English and Chinese are enclosed. Ethics approval will be obtained from the Institutional Review Board, Shanghai first Maternity and Infant health hospital Hospital.

14. Data Handling and record keeping

All data will be stored for three years. A designated research nurse will be responsible for data management including data coding, monitoring and verification.

15. Financing and insurance

The study will be funded somewhere this year pending to be applied.

16. Publication policy

The findings of this study will be submitted for consideration for publication in peer-reviewed scientific journal.

17. Supplements

The study will be conducted in compliance with the Declaration of Helsinki and Good Clinical Practice (ICH-GCP)

18. We certify that the information given is complete and accurate to the best of our knowledge.

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