Effect of Melatonin on Clinical Outcome of Patients with Repeated Cycles after Failed Cycles of *In Vitro* Fertilization and Intracytoplasmic Sperm Injection

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Research Article

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Abstract

Background: Melatonin (MT), as a powerful antioxidant, can effectively ameliorate the *in vitro* development of animal embryos, but few studies have been made on human embryos. Therefore, we investigated whether the application of MT in embryo cultures can improve embryonic development and clinical outcomes in patients with repeated cycles after failed *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles.

Methods: Human immature oocytes from controlled ovarian hyperstimulation cycles were collected for *in vitro* maturation culture and ICSI fertilization. The obtained embryos were cultured *in vitro* in medium containing 0, 10^{-11} , 10^{-9} , 10^{-7} or 10^{-5} M MT, and 10^{-9} M was determined to be the optimal concentration. Subsequently, 140 patients who experienced failed IVF/ICSI cycles received 140 cycles of embryo culture *in vitro* with medium containing 10^{-9} M MT. High-quality blastocysts were collected and cryopreserved for three months before vitrified-warmed embryo transfer. These culture cycles served as the experimental (10^{-9} M) group. The control (0 M) group comprised previous failed cycles. The patients were further divided into subgroups of 1, 2 or ≥ 3 failed cycles. The fertilization and embryo development status were compared.

Results: The fertilization, cleavage, high-quality embryo, blastocyst, and high-quality blastocyst rates of the 10^{-9} M group were significantly higher than 0 M (87.7% vs. 83.6%, $p < 0.01$; 94.1% vs. 90.5%, $p < 0.01$; 58.3% vs. 43.8%, 51.1% vs. 41.8%, 43.4% vs. 22.9%, all $p < 0.0001$). A total of 50 vitrified-warmed cycle transfers were performed. A clinical pregnancy rate of 40% was obtained.

Conclusion: The application of MT to embryo cultures *in vitro* improved embryonic development in patients with repeated cycles after failed IVF/ICSI cycles and obtained good clinical outcomes. The optimal concentration of MT was 10^{-9} M.

Trial registration: Name of the registry: Effect of melatonin on clinical outcome of patients with repeated cycles after failed cycles of in vitro fertilization and intracytoplasmic sperm injection; registration number: ChiCTR2100045552; date of registration: April 19, 2021(Retrospectively registered); URL of trial registry record: www.medresman.org.cn.

Background

Assisted reproductive technology (ART) involves the use of medical methods to artificially manipulate oocytes, sperm, fertilized eggs, and embryos to achieve the goal of conception [1]. Among them, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the core technologies of ART. IVF refers to the natural fertilization of eggs and sperm through artificial methods, and the subsequent *in vitro* culture of early embryos, while ICSI refers to the process of injecting a single sperm into the cytoplasm of an egg using micromanipulation technology to achieve fertilization. *In vitro* fertilization and embryo transfer (IVF-ET) technology has become an important means of infertility treatment since its discovery,

but the risk of poor embryo development with the traditional IVF/ICSI treatment cycle is still unavoidable [2], which leads to pregnancy failure due to poor development of the embryos transferred. Therefore, exploring an efficient technology of embryo culture *in vitro* to obtain high-quality embryos is one of the key features to improve the effect of ART treatment.

Melatonin (MT) is a multifunctional molecule that is mainly produced in the pineal gland [3]. It is a powerful free radical scavenger and antioxidant [4]. MT and its metabolites can directly remove reactive oxygen species (ROS) in cells, activate antioxidant enzymes, increase the expression level and activity of glutathione (GSH), and inhibit pro-oxidant enzymes to reduce cell oxidation injury, which can improve oocyte *in vitro* maturation (IVM) and embryonic development [5]. It has been reported that adding the appropriate amount of MT to IVM and embryo culture medium can significantly improve the developmental potential of oocytes and the *in vitro* development of embryos [6–8]. Through research on human IVF and IVM, Li *et al.* found that the addition of MT to the embryo culture medium can improve the clinical outcomes of IVF and IVM [9]. In 2019, our research team added MT to the IVM medium to culture human immature oocytes from a controlled ovarian hyperstimulation (COH) cycle, which significantly reduced the levels of ROS and Ca^{2+} in oocytes during the IVM process, increased mitochondrial membrane potential, and improved embryo development, which eventually resulted in healthy offspring [10]. The present study was the first attempt to use embryo culture medium supplemented with MT to culture embryos of patients undergoing repeated cycles after failed IVF/ICSI cycles, and aimed to explore whether the application of MT could improve the embryonic development and clinical outcomes of patients with repeated IVF/ICSI cycles.

Materials And Methods

Research patients

A total of 140 patients with failed cycles who visited the Reproductive Medicine Center of the First Affiliated Hospital of Anhui Medical University from June 2020 to November 2020 were selected. Inclusion criteria: (1) at least one IVF/ICSI failed cycle; (2) age of patient ≤ 36 years. Excluding genetic factors of both men and women, the causes of infertility included 18 cases of male-only factors, involving oligoasthenoteratozoospermia; 77 cases of female-only factors, involving 41 cases of sequelae of pelvic inflammatory disease, 26 cases of polycystic ovary syndrome, and 10 cases of endometriosis; 40 cases of combined male and female factors; and 5 cases with unknown causes. Before conducting the study, the partners of all enrolled patients were interviewed and provided signed informed consent.

COH regimen

All patients in this study were treated with gonadotropin hormone-releasing hormone (GnRH) antagonist (flexible regimen) for ovarian stimulation. Recombinant human follicle stimulating hormone (Gn, Gonal F; Serino Barueri, SP, Brazil) was given on the second or third day of the menstrual cycle for ovarian hyperstimulation. After four to five days, the dosage of Gn was adjusted according to the follicle size and hormone level. When the dominant follicle reached 12–14 mm, GnRH antagonist (GnRH-A, Cetrotide,

Merck Serono SA - Geneva, Switzerland) was added until the day of the human chorionic gonadotropin (HCG) injection. When there were two or three follicles with a diameter ≥ 18 mm, HCG (10,000 U; Pregnyl; AESCA Pharma, Austria) was injected, and oocyte retrieval was completed after 36–38 h under the guidance of a transvaginal ultrasound.

Experimental design

Experiment 1

Human immature oocytes obtained from the COH cycle were collected for IVM to obtain mature oocytes (IVM-MII) *in vitro* and perform ICSI insemination. Then, embryos were cultured *in vitro* in medium containing 0, 10^{-11} , 10^{-9} , 10^{-7} or 10^{-5} M MT. Ultimately, 10^{-9} M was determined to be the optimal concentration of MT.

Experiment 2

For the 140 enrolled patients with failed IVF/ICSI cycles, the embryo culture medium containing 10^{-9} M MT was prepared to conduct embryo culture *in vitro* in the subsequent 140 repeated cycles. High-quality blastocysts were collected and cryopreserved. These 140 MT culture cycles were designated as the experimental group (10^{-9} M group), and the control group consisted of the previous failed cycles of the same patients (0 M group). In addition, according to the number of failed cycles, the patients were further divided into subgroups of one, two, or at least three failed cycles, and the fertilization and embryo development status of each subgroup in the 10^{-9} M and 0 M groups were compared and analyzed.

Experiment 3

After 3 months, vitrified-warmed embryo transfer was performed. This study was discussed and approved by the Reproductive Ethics Committee of the First Affiliated Hospital of Anhui Medical University. The experimental process is shown in Fig. 1.

IVM of oocytes from COH cycle

Human immature oocytes with normal morphology from the COH cycle were collected and placed into IVM medium prepared and balanced overnight to culture for 24 h. Subsequently, the IVM-MII oocytes were selected for ICSI insemination, followed by five or six days of embryo culture *in vitro*. The detailed process has been previously reported in published literature [11].

Preparation of the embryo culture medium

An appropriate amount of embryo culture medium (cleavage or blastocyst; Cook, Sydney, Australia) was prepared, and then an appropriate concentration of MT was added. Next, six to eight droplets (30 μ l/drop) were made in a dish with a diameter of 60 mm, and were covered with an appropriate amount of tissue culture oil. Finally, the dish was placed at 37°C with 6% CO₂ and saturated humidity for 18 h of equilibration.

ICSI/IVF insemination

Under a microscope, the cumulus-oocyte complex (COC) in follicular fluid was picked up and placed into balanced fertilization medium (Cook, Sydney, Australia) at 37°C with 6% CO₂ and saturated humidity for 4–6 h of culture *in vitro*, followed by ICSI or IVF insemination. For ICSI insemination, its process has been described in detail in our previously published literature [12]. Following ICSI, the inseminated oocytes were directly transferred into the cleavage culture medium containing MT for embryo culture *in vitro*. For IVF insemination, the COC, which experienced 4–6 h of culture *in vitro*, was transferred into the fertilization medium containing 5×10⁵/ml of grade A and grade B sperm for 6 h of culture at 37°C with 6% CO₂ and saturated humidity. Next, the granule cells around the oocytes were removed and those with the second polar body, which were considered fertilized oocytes, were selected under an inverted microscope. Finally, all fertilized oocytes were transferred into cleavage culture medium with MT for subsequent embryo culture.

Embryo culture

The oocytes undergoing *in vitro* culture in the balanced cleavage culture droplets (one oocyte/drop) were observed based on their fertilization status 14–16 h after insemination, and then the culture was continued under 37°C, 6% CO₂, and saturated humidity. Two days later, all of the formed cleavage embryos were transferred into balanced blastocyte culture droplets (one to three embryos/drop) for additional two or three days of blastocyst culture. Finally, the obtained high-quality blastocysts (see Fig. 2) were selected and cryopreserved in -196°C liquid nitrogen using the vitrification method. In this process, all embryos formed were scored according to Tomas [13] and Gardner [14] scoring standards.

Embryo transfer and pregnancy determination

Three months later, the cryopreserved blastocyst was warmed. According to the patient's age, one or two warmed blastocysts were transferred into the uterus under B-ultrasound guidance. Two weeks after the embryo transfer, serum hCG levels were examined and a biochemical pregnancy was defined as a positive hCG value (≥ 25 IU/L). At seven weeks following embryo transfer, the presence of a gestational sac identified by an ultrasound scan was referred to as a clinical pregnancy. For the detailed operation process of embryo vitrification and warming, please refer to our previously published literature [15].

Statistical analysis

SPSS software version 23.0 was used to perform statistical analysis. The differences in means between continuous variables [female age, male age, basic follicle-stimulating hormone (FSH) level, basic luteinizing hormone (LH) level, basic E2 level, duration infertility, average no.oocyte retrieval and body mass index (BMI)] were expressed as mean $\pm$ SD. The categorical data from the developmental competence (rates of fertilization, cleavage, high-quality cleavage embryo, and high-quality blastocyst) in each group were analyzed using chi-squared test or Fisher's exact test. *P*-values <0.05 were considered statistically significant.

Results

Screening the optimal MT concentration for embryo culture medium

A total of 400 human immature oocytes of 198 COH cycles from 198 patients were collected for IVM culture, and 295 IVM-MII oocytes were obtained. ICSI insemination and embryo culture *in vitro* were performed, and the detailed results are shown in Table 1. There were no significant differences in age, FSH, luteinizing hormone (LH), estradiol (E2), body mass index (BMI), years of infertility, or number of oocytes obtained in each group (see Table 1).

Table 1
Comparison of baseline data of the 5 groups of patients

	0M	10^{-11} M	10^{-9} M	10^{-7} M	10^{-5} M	P-value
Male age (yr)	31.18 ± 3.343	30.85 ± 3.890	30.95 ± 4.214	30.89 ± 3.748	30.24 ± 3.494	> 0.05
Female age (yr)	30.15 ± 3.549	29.33 ± 2.787	29.79 ± 3.361	30.14 ± 2.987	29.17 ± 3.011	> 0.05
Infertility period (yr)	3.200 ± 1.800	3.074 ± 1.796	3.000 ± 1.593	3.000 ± 1.493	3.057 ± 1.598	> 0.05
No. of retrieved oocytes	18.68 ± 8.404	18.44 ± 5.366	18.00 ± 7.191	18.67 ± 6.533	19.13 ± 7.932	> 0.05
FSH	6.663 ± 2.259	7.034 ± 1.911	7.111 ± 1.428	6.465 ± 1.901	7.055 ± 2.362	> 0.05
LH	5.700 ± 2.679	4.959 ± 2.582	5.721 ± 5.465	5.334 ± 3.286	4.965 ± 3.158	> 0.05
E2	167.9 ± 100.4	178.0 ± 94.5	162.1 ± 96.43	177.7 ± 109.5	163.2 ± 94.59	> 0.05
BMI	21.76 ± 2.791	21.66 ± 1.920	22.76 ± 3.680	21.76 ± 2.380	21.91 ± 3.117	> 0.05
Values are presented as mean ± standard deviation.						

As shown in Table 2, after adding 10^{-9} M MT to the embryo culture medium, the fertilization, cleavage, blastocyst and high-quality blastocyst rates of the 10^{-9} M group were significantly different compared to those of the 0 M group ($p < 0.01$). The blastocyst and high-quality blastocyst rates of the 10^{-9} M group were significantly different compared to the 10^{-5} M group ($p < 0.01$, $p < 0.001$), and the blastocyst rate of the 10^{-9} M group was significantly different from that of the 10^{-11} M group ($p < 0.01$). The above data

revealed that the addition of 10^{-9} M MT to the embryo culture could significantly improve the fertilization and *in vitro* development of the fertilized embryo, and 10^{-9} M was the optimal concentration of MT.

Table 2
The effects of different concentrations of MT on embryo development *in vitro*

	0 M	10^{-5} M	10^{-7} M	10^{-9} M	10^{-11} M
IVM-MII (n)	61	58	59	61	56
Fertilization rate (%)	85.2 (52/61)	87.9 (51/58)	83.1 (49/59)	90.2 (55/61) ^a	78.6 (44/56)
Cleavage rate (%)	82.7 (43/52)	88.2 (45/51)	85.7 (42/49)	98.2 (54/55) ^a	84.1 (37/44)
Blastocyst rate (%)	16.3 (7/43)	11.1 (5/45)	21.4 (9/42)	42.6 (23/54) ^{a, c, d}	16.2 (6/37)
High-quality blastocyst rate (%)	4.7 (2/43)	4.4 (2/45)	9.5 (4/42)	24.1 (13/54) ^{a, b, d}	2.7 (1/37)
Datas analyzed using Chi-square or Fisher Exact test. Different symbols within columns and different letters within columns and within rows indicate significant differences. ^a $P < 0.01$, compared with 0 M group ; ^b $P < 0.01$, compared with 10^{-5} M group ; ^c $P < 0.001$, compared with 10^{-5} M group ; ^d $P < 0.01$, compared with 10^{-11} M group.					

Fertilization and subsequent embryo development of oocytes in the 10^{-9} M and 0 M groups

Table 3 showed that in the previous cycle without MT (0 M group), a total of 1838 oocytes were collected, of which 1372 MII oocytes were fertilized by IVF/ICSI, and the fertilization rate was 83.6% (1147/1372). Among the 140 cycles of 140 patients with subsequent added MT (10^{-9} M group), a total of 1658 oocytes were collected, including 1288 MII oocytes, and the fertilization rate after insemination was 87.7% (1129/1288). The fertilization, cleavage, high-quality embryo, blastocyst, and high-quality blastocyst rates in the 10^{-9} M group were significantly different compared to the 0 M group (87.7% vs. 83.6%, $p < 0.01$; 94.1% vs. 90.5%, $p < 0.01$; 58.3% vs. 43.8%, $p < 0.0001$; 51.1% vs. 41.8%, $p < 0.0001$; 43.4% vs. 22.9%, $p < 0.0001$).

Table 3
Comparison of oocyte fertilization and embryo development between the 10^{-9} M group and the 0 M group

	10^{-9} M	0M
Number of cycles	140	240
Number of oocytes	1658	1838
Number of MII	1288	1372
Fertilization rate (%)	1129/1288(87.7) ^a	1147/1372(83.6)
Cleavage rate (%)	1062/1129(94.1) ^a	1038/1147(90.5)
High-quality embryo rate (%)	619/1062(58.3) ^b	455/1038(43.8)
Blastocyst rate (%)	543/1062(51.1) ^b	434/1038(41.8)
High-quality blastocyst rate (%)	461/1062(43.4) ^b	238/1038(22.9)
Data analyzed using chi-squared test or Fisher's exact test.		
Different symbols within columns and different letters within columns and within rows indicate significant difference. ^a $P < 0.01$, compared with the 0 MT group;		
^b $P < 0.0001$, compared with the 0 MT group.		

The result of oocyte fertilization and subsequent embryo development in the 10^{-9} M and 0M groups of patients who experienced 1 failed IVF/ICSI cycle

As shown in Fig. 3, there were 1116 oocytes in the 10^{-9} M group and 1009 oocytes in the 0 M group of patients who experienced a single failed IVF/ICSI cycle. In terms of fertilization rate (90.1% vs. 88.2%) and cleavage rate (94.6% vs. 92.9%), the rates of the 10^{-9} M group were higher than the 0 M group, but these differences were not significant. The high-quality embryo rate (56.8% vs. 39.4%) and high-quality blastocysts rate (42.0% vs. 25.3%) of the 10^{-9} M group was significantly higher than the 0 M group (both $p < 0.0001$).

The result of oocyte fertilization and subsequent embryo development in the 10^{-9} M and 0M groups of patients who experienced 2 failed IVF/ICSI cycles

As shown in Fig. 4, there were a total of 468 oocytes in the 10^{-9} M group and 688 oocytes in the 0 M group of patients who experienced two failed IVF/ICSI cycles. The fertilization, cleavage, high-quality embryo and high-quality blastocyst rates of the 10^{-9} M group were higher than those in the 0 M group. The cleavage, high-quality embryo and high-quality blastocyst rates of the 10^{-9} M group were significantly different from those of the 0 M group (92.2% vs. 85.6%, $p < 0.05$; 51.1% vs. 41.3%, $p < 0.05$, 44.6% vs. 19.9%, $p < 0.0001$).

The result of oocyte fertilization and subsequent embryo development in the 10^{-9} M and 0M groups of patients who experienced ≥ 3 failed IVF/ICSI cycles

As shown in Fig. 5, there were a total of 74 oocytes in the 10^{-9} M group and 141 oocytes in the 0 M group of patients who experienced three or more failed IVF/ICSI cycles. The fertilization, cleavage, high-quality embryo and high-quality blastocyst rates of the 10^{-9} M group were higher than those of the 0 M group. Among these, the high-quality embryo and high-quality blastocyst rates of the 10^{-9} M group were significantly different from those of the 0 M group (45.8% vs. 34.3%, $p < 0.05$, 57.1% vs. 20.9%, $p < 0.0001$).

Clinical transplantation outcomes

By the end of this manuscript submission, the experimental group had conducted 50 cycles of vitrified-warmed embryo transfers. A total of 61 warmed blastocysts were transferred, the average number of transfers was 1.22, and 32 cases achieved biochemical pregnancy. The biochemical pregnancy rate was 64%. Subsequently, on the 30-35th day after embryo transfer, B-ultrasound was used to further monitor the condition of the gestational sac, and 20 cases of clinical pregnancy were confirmed. The clinical pregnancy rate was 40%.

Discussion

In the process of ART treatment, patients encounter many cycles of treatment failure due to poor embryo quality. There are many factors affecting embryo development, such as culture medium [16], CO_2 concentration [17], temperature [17], *in vitro* operations [18, 19], as well as sperm and oocyte quality [20, 21]. It can be considered that during ART treatment, the quality of oocytes is one of the most critical factors that determine the embryo status and clinical outcome. Any factor affecting the quality of the oocyte will affect its fertilization and subsequent embryo development [22], and ultimately the cycle outcome of ART treatment. In this study, an appropriate concentration of MT was added to the embryo culture medium and the embryo development and clinical treatment outcomes of the patients were observed, to understand whether the application of MT has a positive impact on embryo development.

According to research reports, embryos are in a relatively high oxygen environment during *in vitro* culture, which will cause oxidative stress due to the imbalance of the antioxidant defense system, resulting in a large number of ROS produced by oocytes and embryos during development, thus affecting the quality of oocytes and outcome of the embryo development *in vitro* [23]. MT is a highly effective antioxidant with strong antioxidant activity. Animal study has shown that the addition of MT to the embryo culture medium can improve embryo development *in vitro*, and it has also been confirmed that the improvement is concentration-dependent [4]. In 2020, our team found that adding MT to the human IVM medium can improve the IVM outcome of human immature oocytes by promoting mitochondrial function and inhibiting damage due to oxidative stress, and 10^{-5} M was found to be the optimal concentration [10]. Based on the conclusions of the previous studies, two main questions were posed: (1) can adding MT to the culture medium of the human embryos improve the *in vitro* development and clinical outcome of the embryos? And (2) is its effect also correlated to concentration? Therefore, in this study, human immature oocytes in the COH cycle were collected for IVM to obtain IVM-MII oocytes and ICSI insemination was conducted. Next, the fertilized oocytes were cultured *in vitro* in culture medium containing 0, 10^{-11} , 10^{-9} , 10^{-7} or 10^{-5} M MT, and a systematic comparative analysis was performed on the fertilization of oocytes and early embryo development in each group. The fertilization, cleavage, blastocyst and high-quality blastocyst rates of the 10^{-9} M group were all significantly higher than those of the 0 M group. The blastocyst and high-quality blastocyst rates of the 10^{-9} M group were significantly different from those of the 10^{-5} M group, and the blastocyst rate was also significantly different compared to the 10^{-11} M group. In addition, the fertilization, cleavage, blastocyst and high-quality blastocyst rates of the 10^{-9} M group were higher than those of the 10^{-7} M group, but these differences were not significant. It has been reported that the fertilization rate, cleavage rate and total blastocyst cell number of porcine IVF embryos cultured in medium containing 10^{-9} M MT were significantly increased [24]. Wang *et al.* [4] added different concentrations of MT to the culture medium of bovine embryos *in vitro* and found that 10^{-9} M MT could significantly promote embryo development. These results are consistent with the results of the present study. These results showed that the addition of 10^{-9} M MT to human embryo culture medium could significantly improve the fertilization of human oocytes and embryo development *in vitro* obtained after fertilization, and 10^{-9} M was the optimal concentration.

Next, 10^{-9} M was selected as the MT concentration to be added to the embryo culture medium of patients with failed IVF/ICSI cycles for embryo culture *in vitro*. The study found that in terms of fertilization, cleavage, high-quality embryo, blastocyst and high-quality blastocyst rates, the values of the 10^{-9} M group were significantly higher than those of the 0 M group. In this study, further group analysis was also carried out according to the number of failed IVF/ICSI cycles and it was discovered that the application of 10^{-9} M MT to embryo culture *in vitro* could significantly improve embryonic development in the repeated cycle of each group. A large number of studies have found that adding MT to the culture medium can reduce the levels of ROS in oocytes and promote embryonic development of cattle, mice, sheep, and pigs [7, 25–27]. Nakano *et al.* [28] confirmed that the addition of MT during embryo culture *in vitro* can reduce the level of ROS in parthenogenetic embryos and promote embryo development. These

findings are consistent with the results of the current study, suggesting that the improvement of embryonic development in patients with failed IVF/ICSI cycles by MT may be related to the highly effective antioxidant properties of MT. Oxidative stress inhibits oocyte maturation and embryo development, and MT has a strong antioxidant effect, which can resist oxidative stress, maintain the balance of antioxidant system, reduce ROS content in the embryo, and promote gamete maturation and embryo development. In this study, 50 cycles of vitrified-warmed embryo transfers with MT intervention have been performed, and a biochemical pregnancy rate of 64% was achieved, as well as a clinical pregnancy rate of 40%. This result shows that the application of 10^{-9} M MT to embryo culture medium *in vitro* can significantly improve the clinical treatment effect of patients with failed IVF/ICSI cycles, which greatly enhance our confidence in treating such patients, and thereby increases the overall success rate of ART treatment.

In conclusion, the application of MT to embryo culture medium *in vitro* can improve embryonic development for patients with repeated cycles after failed IVF/ICSI cycles and lead to good clinical outcomes. The optimal concentration of MT was 10^{-9} M.

Abbreviations

MT: melatonin; IVF: In vitro fertilization; ICSI: Intracytoplasmic sperm injection; IVM: In vitro maturation; ART: Assisted reproductive technology; IVF-ET: In vitro fertilization and embryo transfer; ROS: Reactive oxygen species; GSH: glutathione; COH: Controlled ovarian hyperstimulation; GnRH: Gonadotropin hormone-releasing hormone; HCG: Human chorionic gonadotropin; GnRH-A: GnRH antagonist; COC: Cumulus-oocyte complex; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; BMI: Body mass index.

Declarations

Ethics approval and consent to participate

This study was approved by the biomedical ethics committee of Anhui Medical University (2015013).

Consent for publication

All authors provided final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no conflicts of interest.

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Authors' contributions

Qi Zhu: Manuscript writing and sample collection; Kaijuan Wang and Chao Zhang: Data collection; Zhaojuan Yu and Bihua Rao: Data analysis; Beili Chen, Huijuan Zou and Weiwei Zou: clinical assessments and experiment conducting; Yunxia Cao: Manuscript editing; Ding Ding and Zhiguo Zhang: Manuscript revising and study design. All authors read and approved the final manuscript.

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Figures

Figure 1

Experimental flow chart. o.n, oocyte number; c.n, cycle number; p.n, patient number.

Figure 2

Representative images of the high-quality blastocysts. Note: (A) High-quality blastocyst 4AA; (B) high-quality blastocyst 4AA; (C) high-quality blastocyst 4AB; (D) high-quality blastocyst 4BA. Scale bar=10uM.

Figure 3

Status of fertilization and early embryo development in patients with one failed IVF/ICSI cycle. Note: A: fertilization; B: cleavage; C: high-quality embryo and D: high-quality blastocyst. (****P < 0.0001).

Figure 4

Status of fertilization and early embryo development in patients with two failed IVF/ICSI cycles. Note: A: fertilization; B: cleavage; C: high-quality embryo and D: high-quality blastocyst. (* $P < 0.05$; **** $P < 0.0001$).

Figure 5

Status of fertilization and early embryo development in patients with one failed IVF/ICSI cycles. Note: A: fertilization; B: cleavage; C: high-quality embryo and D: high-quality blastocyst. (*P < 0.05; ****P < 0.0001).