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GENETICS

Homozygous missense mutation Arg207Cys in the *WEE2* gene causes female infertility and fertilization failure

Xiaoyu Yang[1] · Li Shu[1] · Lingbo Cai[1] · Xueping Sun[1] · Yugui Cui[1] · Jiayin Liu[1]

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Abstract

Purpose To investigate a novel mutation in the *WEE2* gene in a female patient with primary infertility and fertilization failure.
Methods Sanger sequencing was used to detect mutations in *WEE2*. The pathogenicity of the identified variant and its possible effects on the WEE2 protein were evaluated with in silico tools and molecular modeling. We used the calcium ionophore A23187 as a chemical activator of oocytes after intracytoplasmic sperm injection (ICSI).
Results We identified a consanguineous family with a novel homozygous missense mutation in *WEE2* (c.619C>T [p.R207C]). Based on preliminary bioinformatics analysis, we speculate that the novel homozygous missense mutation is pathogenic. ICSI combined with assisted oocyte activation (ICSI-AOA) did not overcome fertilization failure in this patient with *WEE2* mutation.
Conclusions We identified a novel mutation in *WEE2* (c.619C>T [p.R207C]) in a female patient with fertilization failure after ICSI, and we provide evidence that this novel homozygous missense mutation can cause fertilization failure.

Keywords *WEE2* · Mutation · Fertilization failure · Intracytoplasmic sperm injection · Assisted oocyte activation

Introduction

Intracytoplasmic sperm injection (ICSI) has become the most efficient treatment for all forms of male-factor infertility and prior failed fertilization via in vitro fertilization (IVF) [1]. This technique, which was first introduced in 1992, involves injection of a single sperm into the cytoplasm of a mature oocyte [2]. The mean fertilization rate of ICSI has been reported to be approximately 70% [3]. The frequency of total fertilization failure (TFF) after ICSI has been reported to be 1–3% [4]. Oocyte activation deficiency (OAF) appears to be the main cause of total fertilization failure after ICSI when infertile couples have a normal number of oocytes and oval motile sperm [5].

Recently, ICSI in combination with assisted oocyte activation (ICSI-AOA) has been recommended for patients suffering from previous TFF after standard ICSI [6, 7]. Although ICSI-AOA is considered a very efficient technique for overcoming TFF, ICSI-AOA is not efficient in all cases of TFF. Previous studies have shown that ICSI-AOA is more efficient in rescuing sperm-related OAF versus suspected oocyte-related OAF [8, 9]. Analyses of the oocyte-related OAF have often revealed severe cytoplasmic abnormalities of the unfertilized oocytes, which may not be overcome by ICSI-AOA.

The genetic basis of fertilization failure, especially regarding suspected oocyte-related factors, is largely unknown. Only mutations in *PLCZ1*, *TLE6*, and *WEE2* have been shown to cause human fertilization failure following ICSI. *PLCZ1* (MIM: 612399) is widely considered to be the sperm-borne oocyte activation factor that evokes the Ca^{2+} oscillations necessary for initiation of oocyte activation and fertilization [10]. One study recently demonstrated that OAF after ICSI in two infertile brothers was caused by homozygous missense mutation in *PLCZ1* [11]. Mutations in *TLE6* and *WEE2* are reported to be as oocyte-related factors that cause fertilization failure. Alazami et al. identified a mutation in *TLE6* (MIM: 608075) that caused fertilization failure or a very low fertilization rate and arresting zygotes at the one-, two-, and four-cell stage by impairing TLE6 phosphorylation and subcortical maternal complex (SCMC) formation [12]. *WEE2* (MIM: 614084) has important roles in mouse oocytes at different stages. When WEE2 is downregulated, mouse oocytes have been documented to have high MPF activity leading to failure of the metaphase II (MII) exit and the formation of pronuclei

✉ Yugui Cui
cuiygnj@njmu.edu.cn

[1] State Key Laboratory of Reproductive Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

[13]. Sang et al. reported that four patients with *WEE2* mutations underwent seven IVF/ICSI attempts, but none of the oocytes could be fertilized [14].

In the present study, we identified a novel mutation in the *WEE2* gene in a female patient with fertilization failure after ICSI-AOA, and we provide evidence that this *WEE2* missense mutation can cause fertilization failure.

Materials and methods

Patients

The proband (Fig. 1, F1: II-1) is a 27-year-old Chinese woman whose parents were cousins. She is 170-cm tall and weighs 78 kg. She has a 5-year history of primary infertility of an unknown cause. She underwent one failed IVF attempt in AnHui Women and Child Health Care Hospital in 2014. Her husband has normal seminal parameters (110×10^6 spermatozoa per ejaculate, 56% sperm motility, and 8% normal sperm morphology). The couple has normal karyotypes, and no mutations in PLCZ1 were identified in the male partner. The proband has two male siblings aged 20 and 23 years who are not married and have not reproduced. Blood samples were obtained from the affected patient and all available family members. The ethics committee of the First Affiliated Hospital of Nanjing Medical University approved this study. We obtained informed consent from each participant.

ICSI and AOA

Controlled ovarian hyperstimulation and ICSI were carried out as previously described [15]. Oocytes at the MII stage, with the first polar body (PB) extruded, were regarded as mature and were selected for ICSI. Fertilization was evaluated 17 h after the ICSI procedure. Normal fertilization resulted in two pronuclei (PN) and two PB.

ICSI-AOA was performed at least 4–6 h after oocyte retrieval, as previously described with some modifications [16]. After ICSI, oocytes were cultured in Quinn's medium (Sage, USA) for 30 min at 37 °C in a 6% CO_2 air atmosphere. Next, the oocytes were exposed to a calcium ionophore solution containing 10 μmol/L of A23187 (I9657, Sigma-Aldrich, Bornem, Belgium) for 10 min at 37 °C in 6% CO_2 and 5% O_2. Following AOA, the oocytes were rinsed thoroughly in IVF medium (Sage, USA), transferred to cleavage medium and incubated at 37 °C under 6% CO_2 and 5% O_2 conditions.

Sequence analysis of *WEE2*

Genomic DNA was extracted from peripheral blood samples using Tiangen RelaxGene Blood DNA (Tiangene, Beijing, China) according to the manufacturer's protocol. All coding regions of *WEE2* were amplified by PCR using the primers shown in Table 1. The PCR products underwent direct Sanger sequencing in both the forward and reverse directions using an ABI 3100 DNA analyzer (Applied Biosystem, Foster City, CA, USA). Exome Aggregation Consortium (ExAC) Browser (http://exac.broadinstitute.org/) was used to search for the allele frequencies of the variants. Conservation of the affected amino acid residues among species was analyzed through multiple sequence alignment of the human WEE2 protein with its paralog and orthologs using MultiAlin (http://multalin.toulouse.inra.fr/multalin/multalin.html). The pathogenicity of the identified *WEE2* protein variant was assessed using the SIFT algorithm, PolyPhen2, and Mutation Taster. Splicing mutations were evaluated using NNSplice.

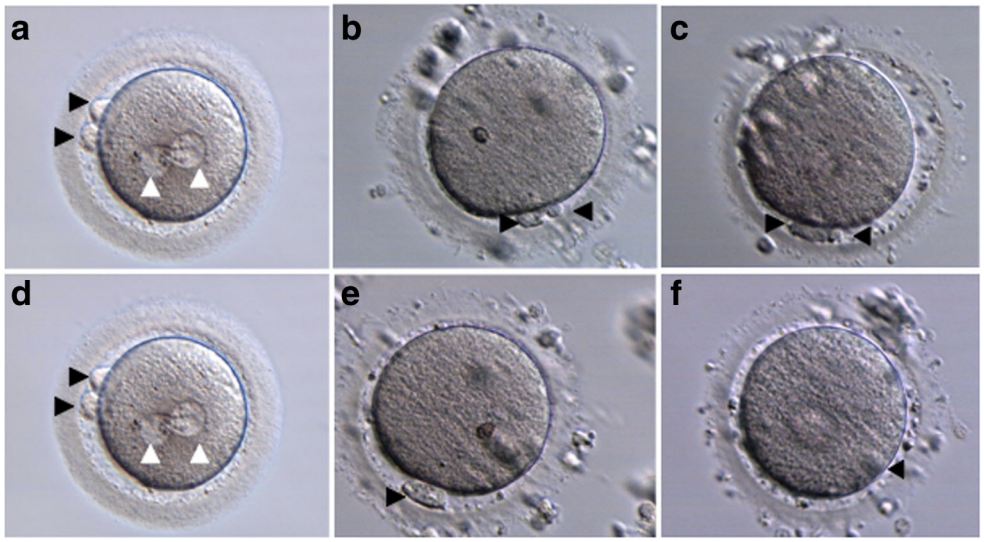

Fig. 1 Morphology of a normal zygote (**a**, **d**) and affected individual oocytes (**b**, **c**, **e**, **f**) on day one after ICSI-AOA. The white arrowhead indicates pronuclei, and the black arrowhead indicates the polar body

Table 1 Genomic PCR primers used to amplify *WEE2* exons for Sanger sequencing

Exon	F/R	Primer sequence (5′–3′)	PCR size (bp)
1	F	TGCTTCTGTAGGTTCACAGCGTTCC	455
	R	GCTTGGCGACCTGCGTCCCTTTT	
2	F	ATGCTATTATGACTTGCCTTTTG	480
	R	TTGCTTATTCTGTCTTATCCTTT	
3	F	CTAGCATAGTTCGGCCTCAATAAAT	373
	R	AGGAGAGAAGGAGAGTAGGAGAAAG	
4	F	ATTGGGGAGATTCTTGCTAC	510
	R	AGCTGGAAAACTCAGAAAGG	
5	F	CCATTGCTAGTAAAGCCTCATAT	427
	R	GACAACAGAAGGGAAAGAAAGAA	
6	F	GAGGTCCTGGAACTAATACCCTG	352
	R	ACCGTCCTTGACACTTGATAGACATA C	
7	F	ATCACTAGCTGTTCAGAAGCGATGT	497
	R	TCATTAGCCAGGAAGCGACTATC	
8	F	TAGCCTTACCAGTTACCATT	360
	R	CTCTCCTATCTTTGGTCTCC	
9	F	GACTCACCCTGCTTGGCATAGTAAC	443
	R	TCTGACCTTGTGGTCCACCCTCC	
10	F	GGAGTTGGCTTGAATGGGAAGAT	360
	R	GTTTCCTTGTGGGCAGGGACCTA	
11	F	AAACCTGCCATGTTGTAT	581
	R	AAGCGATTCTCCTTCCTCA	
12	F	GCCAAGATCCTAAAGCTAGTAAG	231
	R	GGAATCAGCAACAGCAACCTCGT	

Molecular modeling

WEE2 mutations were mapped using PyMOL onto an atomic model of the human WEE2 kinase domain (residues 204–486; PDB: 5VDK; Modeling QMEAN score of − 1.76).

Results

Outcomes of ICSI-AOA

The patient underwent two failed IVF/ICSI attempts (Table 2). In the first IVF cycle, she underwent short-time insemination and early-rescue ICSI at AnHui Women and Child Health Care Hospital as previously described [17, 18]. Twenty-three MII oocytes were retrieved, but none were normally fertilized after early-rescue ICSI. In the second cycle, the patient underwent ICSI-AOA in our reproductive center, but all 16 retrieved MII oocytes were not normally fertilized. None of the 16 unfertilized oocytes had two PN, but nine had two PB (Table 2). The morphology of the representative unfertilized oocytes after ICSI-AOA is shown in Fig. 1.

Sanger sequencing analysis

Our sequencing analysis identified a novel mutation in the *WEE2* gene (NM_001105558). Specifically, P1 (F1: II-1) from a consanguineous family was found to be

Table 2 Clinical characteristics of affected individual

Case	Age (years)	Duration of infertility (years)	Early-rescue ICSI cycle*		ICSI-AOA cycle				
			MIIoocyte	2 PN	MIIoocyte	2 PN	2 PB	1 PB	Dead
P1#	27	5	23	0	16	0	8	5	3

Short-time insemination and early-rescue ICSI were performed in the first cycle, and ICSI-AOA was performed in the second cycle

* No more data about 2 PB could be obtained because early-rescue ICSI was not done in our hospital

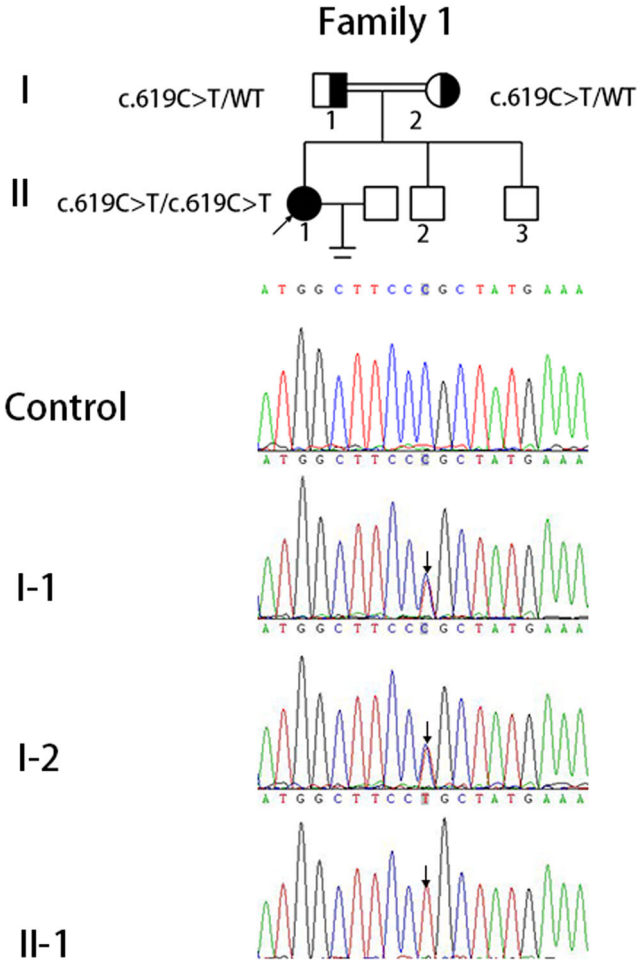

Fig. 2 Pedigree of the family with the *WEE2* mutation. The genes of the parents of P1 were sequenced, revealing that the variants were inherited from her parents. The carrier status of the parents is shown in the pedigree

homozygous for a missense *WEE2* mutation (c.619C>T [p.R207C]) (Fig. 2). The *WEE2* gene was also sequenced in the parents of P1, and the above variant was verified to be inherited from them. With in silico tools, including the SIFT algorithm, PolyPhen-2, Mutation Taster, and NNSplice, we evaluated the predicted effects of the novel mutation (c.619C>T) on protein function (Table 3). Moreover, the novel mutation had a low allele frequency (15/120, 232) in the ExAC dataset and its newly released Genome Aggregation Database (gnomAD), and the homozygote frequency was zero (Table 3). The novel *WEE2* mutation (c.619C>T) appears to be present among Europeans (14/66,518) and Africans (1/9718) but has never been detected in East Asians (0/8514) (http://exac.broadinstitute.org/variant). These observations suggest that the novel homozygous *WEE2* missense mutation (c.619C>T) is pathogenic. Specific information on the mutations is shown in Table 3. The position of the mutation, along with its conservation in different species, is shown in Fig. 3a.

Table 3 Effects of novel WEE2 mutation predicted using in silico tools

Chromosome 7 co-ordinates[a]	cDNA alteration	Amino acid alteration	Exon	Mutation	ExAC allele frequency	ExAC homozygotes frequency	gnomAD allele frequency	gnomAD homozygotes frequency	NN splice	SIFT	PolyPhen-2	Mutation taster
141418905C>T	c.619 C>T	p. R207C	4	Missense	15/120232	0/120232	42/280578	0/280578	–	0.01 (D)[b]	1 (P)[b]	1.000 (D)[b]

The letter P in the brackets indicates possibly damaging

[a] All data are based on GRCh37/hg19

[b] The letter D in the brackets indicates deleterious

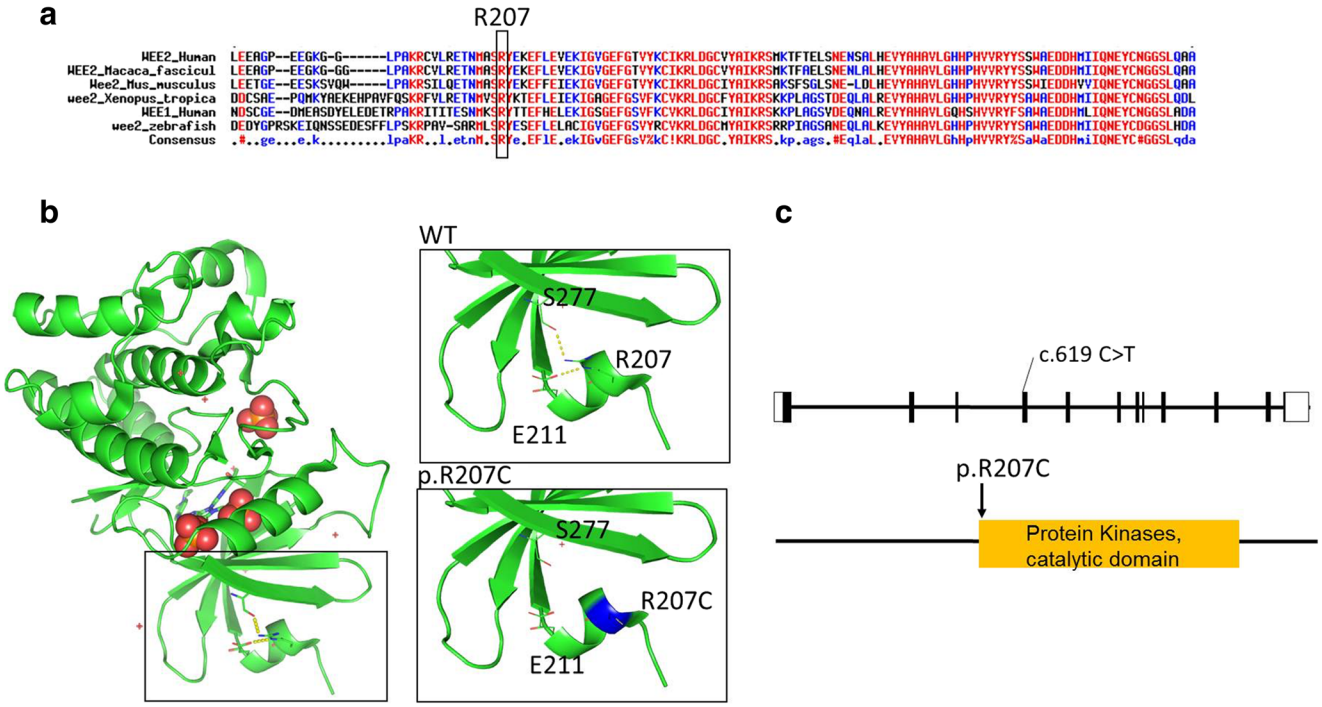

Fig. 3 Prediction of the effects of the mutations on protein conformation. **a** The R207 residue (indicated by the black box) is conserved across species and WEE1. **b** The secondary structure of the kinase domain of WEE2 (residues 204–486) is shown. The right lower panel shows a magnified view of the outlined region containing the conserved residues and the mutant R207 residue. The corresponding wild-type (WT) (R207) form of this outlined region is shown in the right upper panel. **c** The position of the novel mutation identified in this study is indicated in the genomic structure of WEE2 and in the structure of its protein

Prediction of the effects of the mutations on protein conformation

The R207 residue, together with nearby residues, is highly conserved across species and WEE1 based on MultiAlin analysis (Fig. 3a). Notably, the R207 residue, which is located in an α-helix, may form hydrogen bonds with E211 and S277, which are highly conserved across species (Fig. 3b). The p.R207C change in WEE2 may destabilize the local environment by breaking the hydrogen bonds between these residues. Simultaneously, destruction of hydrogen bonds may lead to changes in the α-helix configuration.

Discussion

Human fertilization involves many biochemical changes, including sperm capacitation, the acrosome reaction, sperm-egg binding and fusion, oocyte activation, and pronuclear formation [19, 20]. The main causes of failed fertilization after ICSI are OAF and Ca^{2+} oscillation loss [21] as more than 80% of these oocytes are found to contain a sperm, and OAF is observed in approximately 40% of unfertilized oocytes undergoing ICSI [4].

WEE2 is a crucial oocyte-specific kinase that participates in maintaining meiotic arrest by inhibiting the phosphorylation of Cdc2, which inactivates maturation-promoting factor (MPF), a complex of Cdc2 and cyclin B [13, 22]. *WEE2* mutations in human were identified in four female patients with fertilization failure, including c.700G>C, c.1473dupA, c.220_223delAAAG, c.1006_1007insTA. These mutations proved to be truncated or loss of function, reducing the amount of WEE2 protein and impairing the phosphorylation of WEE2 and Cdc2 [14].

In the present study, we identified a novel homozygous mutation (c.619C>T [p.R207C]) in *WEE2*, which is responsible for maintaining meiotic arrest in mice [23]. Based on the molecular model [24], the highly conserved R207 residue may form hydrogen bonds with E211 and S277 to maintain the stability of the protein conformation. The p.R207C change in WEE2 may destabilize the local environment or disrupt the function of the protein kinase domain by destroying the hydrogen bonds between these residues. Simultaneously, destruction of hydrogen bonds may result in changes in the α-helix configuration. Functional experiments are required to determine whether this variant (c.619C>T) significantly reduces the protein level and impairs the phosphorylation level of WEE2 and Cdc2.

WEE2 is necessary to initiate MPF inactivation by inactivating Cdc2 for exit from MII in mouse oocyte. *WEE2*-knockdown mouse oocytes have high MPF activity leading to failing to form pronuclei and extrude the second PB [13]. MPF activity is also high in *WEE2*-mutated human

oocytes by inactivating Cdc2, which leads to failure of formation of pronuclei and fertilization failure in these patients [14]. But the human oocytes with *WEE2* mutations can be activated by sperm and complete the second meiosis (extrusion the second PB); the exact pathogenic mechanism remains unknown.

Current strategies to improve the outcomes of some cases of fertilization failure after ICSI are AOA, which involves the use of various mechanical, electrical, or chemical stimuli [25, 26]. Chemical activation with A23187, ionomycin and strontium chloride ($SrCl_2$) is the commonly used AOA procedure and leads to increased intracellular Ca^{2+} levels in the oocyte [27–29]. Several studies have reported that ICSI-AOA improves fertilization in patients with TFF or with poor fertilization in previous ICSI cycles [9, 28, 30, 31]. A recent review suggested that human recombinant PLCζ, as a novel therapeutic agent, may rescue activation deficiency when injected into the oocyte because recombinant PLCζ promotes calcium oscillations in a dose-dependent manner [25]. However, ICSI-AOA does not always overcome human fertilization failure, especially in cases of suspected oocyte-related activation deficiency.

Our results showed that ICSI-AOA could not rescue fertilization failure in a female patient with a WEE2 mutation; none of the 16 unfertilized oocytes had two PN, but eight had two PB. Two PB were observed in half of the abnormal fertilized oocytes, similar to the results described by Sang et al. in the literature [14]. However, more cases are needed to verify such conclusions. In our center, fertilization failure usually requires observation and verification by more than two embryologists. The embryologists analyzed and evaluated oocytes before and after fertilization, confirmed the changes of the first and second polar bodies, and excluded polar body fragmentation and the fragmentation of perivitelline space.

WEE2-knockdown mouse oocytes have been shown to exhibit normal cortical granule release, which is one of the earliest Ca^{2+}-induced events, suggesting that Ca^{2+} signals are not impaired [13]. We hypothesize that Ca^{2+} oscillations after sperm-egg fusion are normal in patients with the *WEE2* mutation, but the Ca^{2+} signal cannot drive resumption of the cell cycle due to increased MPF activity caused by the *WEE2* mutation. This assumption may explain why ICSI-AOA could not overcome fertilization failure in this couple. Unnecessary repeated treatment with ICSI or ICSI-AOA can be avoided with a genetic diagnosis that is achieved by testing for *WEE2* mutations in female patients.

Affected individuals need efficient and safe treatment options. Sang et al. reported that injection of *WEE2* cRNA into oocytes from patients with *WEE2* mutations led to successful fertilization and blastocyst formation in vitro, suggesting that this strategy may become an efficient therapy in the near future [14]. We should be very careful when using *WEE2* cRNA for ICSI treatment. Since the amount of protein that will be transcribed in the oocyte after injecting *WEE2* cRNA is uncertain, the quality of embryo may be affected, potentially endangering the health of offspring. In addition, WEE2 cRNA itself may affect embryonic development. Egg donation is also one of the treatment options for the couple.

Conclusions

In summary, we identified one novel mutation in the *WEE2* gene that possibly led to human fertilization failure, and ICSI-AOA did not overcome this related fertilization failure.

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Compliance with ethical standards

The ethics committee of the First Affiliated Hospital of Nanjing Medical University approved this study. We obtained informed consent from each participant.

Conflict of interest The authors declare that they have no conflict of interest.

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