Pregnancy with oocytes characterized by narrow perivitelline space and heterogeneous zona pellucida: is intracytoplasmic sperm injection necessary?

Mei Li · Shui-Ying Ma · Hui-Jun Yang · Ke-Liang Wu ·
Wan-Xia Zhong · Guan-Ling Yu · Zi-Jiang Chen

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Abstract

Purpose This retrospective study analyzed fertilization protocols and pregnancy outcomes for oocytes with narrow perivitelline space and heterogeneous zona pellucid (NPVS/HZP).

Methods In 63 in-vitro fertilization cycles filled with NPVS/HZP oocytes (abnormal oocytes group) and 521 cycles with normal oocytes (normal oocytes group), major clinical and laboratory parameters were recorded and compared in different fertilization cycles (conventional IVF cycles, rescue ICSI cycles, and traditional ICSI cycles).

Results NPVS/HZP oocytes meant lower MII oocytes rates in both IVF and ICSI cycles compared with normal oocytes

($p < 0.05$). The 2PN rates for abnormal oocytes were significantly lower than those for normal oocytes in both conventional IVF cycles (58.8 % VS 71.3 %, $P < 0.05$) and rescue ICSI cycles (58.0 % VS 78.0 %, $P = 0.0000$). The high-quality embryo rates in normal oocytes groups were significantly higher than those in abnormal oocytes groups in different fertilization cycles (52.2 % VS 35.0 %, $P < 0.01$; 42.9 % VS 23.9 %, $P < 0.001$; 50.6 % VS 31.0 %, $P = 0.0000$, respectively). No clinical pregnancy was obtained from abnormal oocytes in 11 conventional IVF cycles. The clinical pregnancy rates in rescue ICSI and traditional ICSI cycles were comparatively lower in abnormal oocytes groups, but there was no significant difference as compared with normal oocytes groups (35.0 % VS 48.1 % and 26.7 % VS 50.7 %, $P > 0.05$, respectively).

Conclusions Retrieval of oocytes characterized by NPVS/PZP from cycle to cycle was one of the reasons for obscure infertility. ICSI may be the right way to avoid fertilization failure and get pregnancy in women with NPVS/HZP oocytes.

Keywords Abnormal oocytes · Perivitelline space · Zona pellucid · Fertilization · Pregnancy

Capsule There is usually no relationship between the oocytes morphology and fertilization protocol during ART treatment. Oocytes with narrow perivitelline space (PVS) and heterogeneous zona pellucida (ZP) in previous assisted reproductive technology (ART) treatment cycle usually resulted in repeated in vitro fertilization (IVF) failure. Study on cycle outcome for these abnormal oocytes may provide useful information for further treatment and research.

M. Li · S.-Y. Ma · H.-J. Yang · K.-L. Wu · W.-X. Zhong · G.-L. Yu ·
Z.-J. Chen (✉)
Center for Reproductive Medicine, Provincial Hospital Affiliated to
Shandong University, Jinan, People's Republic of China
e-mail: chenzejjiang@hotmail.com

M. Li · S.-Y. Ma · H.-J. Yang · K.-L. Wu · W.-X. Zhong · G.-L. Yu ·
Z.-J. Chen
National Research Center for Assisted Reproductive Technology and
Reproductive Genetics, Jinan, People's Republic of China

M. Li · S.-Y. Ma · H.-J. Yang · K.-L. Wu · W.-X. Zhong · G.-L. Yu ·
Z.-J. Chen
The Key Laboratory for Reproductive Endocrinology of Ministry of
Education, Jinan, People's Republic of China

M. Li · S.-Y. Ma · H.-J. Yang · K.-L. Wu · W.-X. Zhong · G.-L. Yu ·
Z.-J. Chen
Shandong Provincial Key Laboratory of Reproductive Medicine,
Jinan, People's Republic of China

Introduction

Normal features of a healthy mature oocyte at metaphase II include a round even shape, light-coloured cytoplasm with homogenous granularity, a small perivitelline space (PVS) without debris, and a transparent zona pellucida (ZP). A majority (60 %–70 %) of the oocytes retrieved from stimulated cycles exhibit one or more abnormal morphologic characteristics [1]. Clinical studies showed that there was no relationship between the oocytes morphology and fertilization results, especially the extracytoplasmic anomalies [2–8].

However, analysis of the literature showed that the effect of oocyte morphologic deviations on intracytoplasmic sperm injection (ICSI) outcome remained controversial [1, 6, 9]. Slight dysmorphisms such as darkness of the ZP and cytoplasm don't affect the fertilization and development potential of the oocytes [5, 6, 10]. Repetition of specific oocytes dysmorphisms such as organelle clustering or large cytoplasmic vacuoles from cycle to cycle had an adverse impact on ICSI outcome and consequently resulted in low implantation rate [6, 11, 12]. Some repeated oocytes dysmorphisms even resulted in fertilization failure though ICSI had been used [13]. The severe dysmorphology of the oocytes reported was intracytoplasmic anomalies usually. Though many studies paid attention to abnormal ZP or PVS [14], such severe dysmorphology as interlocking of extremely narrow PVS and heterogeneous ZP (NPVS/HZP) has never been reported.

The PVS of mammalian oocytes contains a hyaluronan-rich extracellular matrix prior to fertilization. The PVS around germinal vesicle containing oocytes is relatively small and difficult to visualize with light microscopy [15]. With the development of the oocytes, the PVS become visible and the first polar body is easy to visualize. Oocytes with large PVS during ART treatment were usually reported with lower fertilization rate [16, 17]. Narrow PVS was usually observed in immature oocytes, and it was rare that all oocytes retrieved were accompanied with narrow PVS in one in vitro fertilization (IVF) cycle.

The ZP is an extracellular translucent matrix composed of long, crosslinked filaments, which first appears during oocyte growth and increases in thickness as oocytes increase in diameter. The round transparent ZP around oocytes from different women may present different morphologies in IVF cycles. ZP morphological abnormalities reported were centered in shape or thickness variety [18–22]. No previous report has been focused on the ZP texture under inverted light microscopy. ZP plays a critical role in fertilization by acting as a “docking site” for binding of spermatozoa followed by induction of the acrosome reaction in the zona bound sperm. Any disturbance in ZP morphology or texture may lead to abnormal fertilization result. Narrow PVS and heterogeneous ZP were always concurrence with the abnormal oocytes in our study. Data collected in our lab showed that oocytes with these characteristics could not obtain good fertilization results during conventional IVF treatment, and usually resulted in pregnancy failure.

In recent years, early rescue ICSI had been widely used for preventing of fertilization failure. Oocytes have been observed to be fertilized 2–4 h after exposure to spermatozoa, and the second polar body is released in ~90 % of fertilized oocytes by 6 h [23, 24]. Rescue ICSI after 6 h post-insemination gave better fertilization, pregnancy and implantation rates compared with rescue ICSI after 22 h when oocytes have become aged [25]. Xiong's report indicated that cumulus cells removal

at 6 h of insemination was a relatively safe operation with comparable normal fertilization rate and clinical pregnancy rate [26]. Short term co-culture for 4 h after insemination was also used for preventing complete fertilization failure in patients with borderline semen [27]. In our center, early rescue ICSI was performed after 5 h post-insemination for none of the MII oocytes had second polar body extrusion, which meant complete fertilization failure. Because of lower fertilization rate for NPVS/HZP oocytes during conventional IVF cycles, early rescue ICSI was usually performed for prevention of full fertilization failure.

An analysis of the lab and clinical data about these abnormal oocytes was reported in this paper, which may provide insight into further research and treatment. For these women with abnormal oocytes, all transferred embryos arose from NPVS/HZP oocytes. Therefore, the impact of dysmorphology could be assessed not just in terms of their effect on early stages of embryonic development in the IVF laboratory, but also on implantation.

Materials and methods

Study population and participants

This was a retrospective study on severe abnormal oocytes during ART treatments in Provincial Hospital Affiliated to Shandong University between August 2007 and September 2011. During data collection, all of IVF cycles were given short term insemination. Oocyte morphology including polar body extrusion was evaluated for every oocyte after cumulus denudation 5 h after insemination. For traditional ICSI cycles, oocytes nuclear maturation and characteristics were evaluated after cumulus removal before ICSI procedure. There were about 105 IVF/ICSI cycles filled with abnormal oocytes characterized by NPVS/HZP. No normal oocyte without those characteristics could be found in those cycles. In this paper, for data consistence, only the cycles with gonadotropin-releasing hormone agonist down-regulation protocol (GnRH agonist long protocol) were analyzed and compared. Sixty-three IVF/ICSI cycles with all retrieved oocytes characterized by NPVS/HZP were involved in this study (abnormal oocytes group). Another 521 IVF/ICSI cycles without such abnormal oocytes retrieved on the same day of those abnormal oocyte group was included as the normal control group (normal oocytes group), which were stimulated with GnRH agonist long protocol also. Cycles where sperm retrieval required epididymal or testicular puncture were eliminated due to poor recovery of motile sperm concentrations in both groups. This study was approved by the ethics committee at Provincial Hospital Affiliated to Shandong University.

Stimulation protocol and oocyte retrieval

Controlled ovarian hyperstimulation (COH) protocol for all women was achieved using individualized protocols of recombinant follicle stimulating hormone (rFSH; Gonal F, Serono, Switzerland) after gonadotropin-releasing hormone agonist (GnRH; Ferring, Germany) down-regulation (GnRH agonist long protocol) [28]. A 10000-IU dose of human chorionic gonadotropin (hCG; Lizhu, China) were intramuscularly administered when at least one follicle in the two ovaries reached a diameter of 18–20 mm. Oocyte retrieval was performed by transvaginal ultrasound-guided follicles aspiration 35–37 h later.

Classification criteria for abnormal oocytes and normal oocytes

All the oocytes and embryos in the abnormal oocytes group had the same characteristics as follows, while no such characteristics could be found in normal oocytes group.

- (1) The oocyte–corona–cumulus complex (OCCC) was usually described as “ova nuda”, which had less granular and cumulus cells around the oocytes compared with the normal OCCC on the oocytes retrieval day (Fig. 1a and b).
- (2) After cumulus denudation, the heterogeneous ZP looked bright, vitreous, lack of gradation, and the outer edge was irregular. The PVS was too narrow to be differentiated. The polar body was invisible or very flat, or just a flaw at the edge of oocyte cytoplasm, which was difficult to distinguish. The decision of oocytes maturity stage was discerned under inverted microscopy (Fig. 1c and d).
- (3) It was unresisted during ICSI being performed on these abnormal oocytes and no crease left after injection compared with the normal oocytes (Fig. 1e and f).
- (4) Morphology of zygotes derived from abnormal oocytes looked similar to those from normal ones except no obvious PVS and clear polar bodies (Fig. 1g and h). There was no clear blastomere interval in embryos from abnormal oocytes compared with embryos from normal oocytes (Fig. 1i, j, k and l).

There was no variation among operators in classifying the oocytes. All the abnormal oocytes cycles were identified by the lab director and another two experienced embryologists.

Semen preparation

Semen samples were collected by masturbation after 3–7 days of abstinence and prepared by discontinuous density gradient centrifugation. Sperm preparation was carried out with use of 45 % and 90 % Sperm Grad solution (Vitrolife, Sweden). The

pellet was layered with G-IVF medium (Vitrolife, Sweden) supplemented with 5 mg/mL HSA for swim-up.

Oocyte insemination and embryo culture

All of the women were given IVF protocol in the first ART treatment cycle unless accompanied with severe male-factor infertility. If the number of forward movement sperm collected from the husband was less than 5 million on the day of insemination, these women would be given traditional ICSI treatment. Another reason for ICSI decision was for experienced fertilization failure in previous IVF cycles.

Oocytes were inseminated with prepared spermatozoa in 2 to 4 h after oocytes retrieval in all cycles. For traditional ICSI procedure, oocytes nuclear maturation and characteristics were evaluated after cumulus removal by brief exposure to 80-IU/mL hyaluronidase (Sigma Chemical Co., St. Louis, MO) in HEPES-buffered medium. For IVF cycles, short-term co-culture with sperm was performed. Cumulus enclosed oocytes were denuded after 4 to 5 h co-culture with spermatozoa in the insemination media. Evaluation of oocytes nuclear maturation and fertilization deduction were performed after oocytes cumulus removal. MII oocyte was identified by at least one polar body exist. Fertilization estimation was confirmed by the extrusion of the second polar body.

For IVF cycles, early rescue ICSI was performed if none of the MII oocytes had second polar body extrusion till 5 h after insemination, which was named rescue ICSI cycles. If the second polar body appeared in any of the MII oocyte, or there was no visible mature oocyte, no rescue ICSI was carried out, which was named conventional IVF cycles. Fertilization and embryo culture results in different fertilization cycles were analyzed separately.

Embryo culture was carried out in equilibrated 50- μ L droplets of embryo culture media G-1 (Vitrolife, Sweden) under oil at 37 °C in a humidified atmosphere of 6 % CO₂. Normal fertilization was assessed at 16 to 20 h (day 1) after insemination by the appearance of two pronuclei (2PN). Appearance of one pronucleus or more than two pronuclei in the cytoplasm was defined as abnormal fertilization. Fertilization rate calculation based on the zygotes with normal fertilization and abnormal fertilization. Embryo quality was assessed after another 48 h culture in G-1 for normal fertilized zygotes. According to Puissant's standard for embryo grades, seven to ten blastomeres embryos on day 3 and three to five blastomere embryos on day 2 with fragmentation $\leq$ 30 % and regular or slightly irregular blastomeres were defined to be high-quality embryos. High-quality embryos on day 2 were only counted for day 2 embryo transfer. Poor-quality embryos not fit for transfer and cryopreservation were those embryos with less than three blastomeres on day 3 or any cleaved embryos without discernible blastomeres.

Embryos transfer and pregnancy detection

Embryo transfer was performed after 48 h (day 2) or 72 h (day 3) of embryo culture. If there was no more than two embryos on day 2, embryo transfer would be performed immediately. No more than 2 embryos were transferred to woman who was <35 years old in the first IVF cycle. No more than 3 embryos were transferred to women ≥ 35 years old or who had experienced embryos transfer. If there was no embryo or only poor-quality embryos got in whole IVF/ICSI cycle, the embryo transfer would be cancelled, which was named abandoned transfer cycle. Biochemical pregnancies were confirmed by measuring serum beta-hCG levels 14 days after embryo transfer. Clinical pregnancy was determined by ultrasound demonstration of a gestational sac at 7 weeks.

Statistical analysis

Age, infertility duration, estradiol (E2) level on the day of hCG injection, number of oocytes retrieved, MII oocyte rate, fertilization rate, 2PN rate, high-quality embryo rate, abandoned transfer rate and clinical pregnancy rate and implantation rate were recorded and compared as well. Other factors related to fertilization protocol were also compared between normal and abnormal oocytes group. Statistics analysis was performed by *t* test and chi-square test. SPSS (version 16.0) was used for all statistical analyses. Values were considered significant when $P < 0.05$.

Results

Overall

During data collection, there were about 105 IVF/ICSI cycles filled with abnormal oocytes characterized by NPVS/HZP in about total 17000 IVF/ICSI cycles (about 0.6 %). It was a small probability event, but it embarrassed some encountered women actually. Many women had to repeat different COH cycles with same characteristic oocytes retrieved every time, and some gave up for disappointed results. Among these abnormal oocytes cycles, 63 cycles were given GnRH agonist long protocol and 42 cycles were given other ovarian stimulation protocols.

Sixty-three cycles filled with abnormal oocytes for 49 women and 521 normal oocytes cycles for 521 women were analyzed. The women in the abnormal oocytes group had to accept repeated COH cycles (1.3 ± 0.6 per woman) for infertility treatment compared with normal oocytes group in this study. Infertility duration for women in abnormal oocytes group was significantly longer than that in normal oocytes group (6.4 ± 3.3 VS 4.3 ± 2.6 , $p < 0.05$) (Table 1). The similar tendency was also presented in different fertilization cycles

Fig. 1 Representative images of the abnormal and normal oocytes and embryos under light inverted microscopy. **a** The abnormal oocyte–corona-cumulus complex with less granular cumulus around the abnormal oocyte, and **(b)** the normal one with much granular cumulus surrounded. **c** The denuded oocyte with narrow perivitelline space (PVS), heterogeneous zona pellucida (ZP) with less smooth outer edge, and hard to distinguished polar body compared with **(d)** the normal oocyte. **e** No resistance during ICSI being performed and no crease left after ICSI for abnormal oocyte compared with **(f)** the normal oocyte. **g, h** The morphology of two pronuclear (2PN) zygotes from abnormal and normal oocytes looked similar without consideration of PVS. **i, k** The cleaved embryos at 4–8 cell stage without clear blastomere interval for abnormal oocytes and **(j, l)** normal 4–8-cell embryos Original magnification: 200 $\times$

(Table 2, Table 3). 81.6 % (40/49) of the women in the abnormal oocytes group was diagnosed as primary infertility, which was significantly higher than that in the normal oocytes group (49.5 %, 258/521) ($p = 0.0000$). No. of ICSI treatment cycles in abnormal oocytes group (23/63, 36.5 %) was significantly higher than that in normal oocytes group (92/521, 17.7 %) ($p < 0.001$), which was the result of repeated previous fertilization failure (Table 1). Most of ICSI cycles in abnormal oocytes group were for previous fertilization failure (18/23, 78.3 %). In the IVF treatment cycles, 72.5 % (29/40) cycles in abnormal oocytes group were given rescue ICSI for no clear second polar body extrusion, which was significantly higher than 7.0 % (30/429) in normal oocytes group (Table 2).

Women's age, number of oocytes retrieved, E2 levels on the day of hCG administration were similar between the normal oocytes groups and abnormal oocytes groups in different fertilization cycles ($p > 0.05$) (Tables 2 and 3).

Fertilization, embryo development and pregnancy result

For conventional IVF cycles during IVF treatment, the rates of MII oocytes, two polar bodies, and fertilization in the abnormal oocytes group were significantly lower than those in normal oocytes group ($P = 0.0000$) (Table 2). The rate of 2PN and high-quality embryo in conventional IVF cycles for abnormal oocytes were also significantly lower than those for normal oocytes (58.8 % VS 71.3 %, $P < 0.05$; 35.0 % VS 52.2 %, $P < 0.01$). As a result, there was a high abandoned transfer rate per retrieval cycles and no clinical pregnancy was obtained after 3 cycles of embryo transfer in 11 conventional IVF cycles (0/3) in abnormal oocytes group, which was significantly lower than that in the normal oocytes group (165/322, 51.2 %) (Table 2).

MIIOocytes rates in the rescue ICSI cycles in the abnormal oocytes group and normal oocytes group were similar (80.8 % VS 83.3 %, $P > 0.05$). The higher MII oocytes rate in the rescue ICSI cycles as compared with conventional IVF cycles for abnormal oocytes might be traced back to that only cycles

Table 1 Basal information for abnormal oocytes group and normal oocytes group

Variable	Abnormal oocytes group	Normal oocytes group
Total no. of COH cycles	63	521
No. of women enclosed	49	521
No. of COH cycles per women	1.3±0.6	1±0
Average infertility duration(years)	6.4±3.3 ^a	4.3±2.6
The rate of women diagnosed as primary infertility (n)	81.6 % (40/49) ^c	49.5 % (258/521)
The rate of ICSI treatment cycles ^Δ (n)	36.5 % (23/63) ^b	17.7 % (92/521)
The rate of previous fertilization failure for ICSI	78.3 % (18/23) ^c	18.5 % (17/92)

Δ ICSI treatment cycles included cycles intended ICSI for severe male-factor infertility or previous fertilization failure in previous IVF cycles

^a $P<0.05$

^b $P<0.001$

^c $P=0.0000$

P -value <0.05 was considered statistically significant

with MII oocytes were given rescue ICSI. Though rescue ICSI had been performed on the oocytes without two polar bodies, the rates of fertilization, 2PN, and high quality embryo in rescue ICSI cycles for abnormal oocytes were significantly lower than those for normal oocytes (68.0 % VS 87.5 %, $P=0.0000$; 58.0 % VS 78.0 %, $P=0.0000$; 23.9 % VS 42.9 %, $P=0.0000$).

Table 2 Clinical and lab data for abnormal oocytes group and normal oocytes group in IVF cycles

Variable	Abnormal oocytes group (n=40)		Normal oocytes group (n=429)	
	Conventional IVF cycles (n=11) (A)	Rescue ICSI cycles ^Δ (n=29) (B)	Conventional IVF cycles (n=399) (C)	Rescue ICSI cycles ^Δ (n=30) (D)
Fertilization protocol				
Rescue ICSI cycles rate(n)	—	72.5 % (29/40) ^d	—	7.0 % (30/429)
Average ages(years)of the patients	30.6±4.2	30.3±4.4	31.8±4.1	30.6±3.7
Average infertility duration(years)	6.7±3.3 ^a	6.2±3.5 ^a	4.3±2.9	4.4±3.6
E2 level on the day of HCG	3760.1±2598.8	3739.3±1937.0	4396.2±2416.6	4123.3±2041.8
Number of oocytes retrieved	8.4±6.4	9.9±5.0	11.9±6.5	12.2±7.0
MIIOocyte rate(n) ^{ΔΔ}	37.0 % (34/92) ^d	80.8 % (231/286)	86.0 % (4083/4748)	83.3 % (305/366)
Oocyte with two polar bodies rate(n)	30.4 % (28/92) ^d	0	63.4 % (3011/4748)	0
Fertilization rate(n)	70.6 % (24/34) ^d	68.0 % (157/231) ^d	83.4 % (3404/4083)	87.5 % (267/305)
2PN rate(n)	58.8 % (20/34) ^a	58.0 % (134/231) ^d	71.3 % (2912/4083)	78.0 % (238/305)
High-quality embryo rate(n)	35.0 % (7/20) ^b	23.9 % (32/134) ^c	52.2 % (1520/2912)	42.9 % (102/238)
Abandoned transfer rate per retrieval cycles(n)	72.7 % (8/11) ^d	31.0 % (9/29) ^a	2.0 % (8/399)	3.3 % (1/30)
Clinical pregnancy rate per transfer cycles (n)	0(0/3)	35.0 % (7/20)	51.2 % (165/322)	48.1 % (13/27)
Implantation rate per transfer cycles (n)	0(0/4)	25.7 % (9/35)	31.8 % (225/708)	28.3 % (17/60)

Variables were compared in the same fertilization protocol cycles between abnormal oocytes group and normal oocytes group: A versus C; B versus D

^Δ rescue ICSI cycles meant that there was no visible second polar body extrusion for any of MII oocyte at 5 h after conventional IVF

^{ΔΔ} MIIOocyte rate was evaluated when cumulus enclosed oocytes were denuded after 5 h co-culture with spermatozoa in the insemination media

^a $P<0.05$

^b $P<0.01$

^c $P<0.001$

^d $P=0.0000$

P -value <0.05 was considered statistically significant

$P<0.001$, respectively). A high abandoned transfer rate per retrieval cycles was also got in the rescue ICSI cycles for abnormal oocytes (31.0 % VS 3.3 %, $P<0.05$). When it comes to the clinical pregnancy rate and implantation rate in rescue ICSI cycles, no significant difference between abnormal oocytes group and normal oocytes group was found (35.0 % VS 48.1 % and 25.7 % VS 28.3 %, $P>0.05$, respectively) (Table 2). Another interesting phenomenon showed that there was no improvement in fertilization rate and high-quality embryo rate after rescue ICSI being performed, while there was great improvement in clinical pregnancy rate and implantation rate in rescue ICSI group (Table 2). The main reason was that D2 embryos were evaluated usually in conventional IVF cycles for abnormal oocytes for limited embryos got. If these embryos were cultured to D3, most of those embryos would not develop into high-quality embryos.

In traditional ICSI cycles, the MII oocytes rate in the abnormal oocytes group was significantly lower than normal oocytes group (70.3 % VS 85.9 %, $P=0.0000$). The 2PN rate was similar between abnormal oocytes group and normal oocytes group after traditional ICSI treatment ($P>0.05$). As a result of significant lower high-quality embryo rate (31.0 % VS 50.6 %, $P=0.0000$), abandoned transfer rate in abnormal oocytes group was significantly higher than that in normal oocytes group in traditional ICSI cycles (30.4 % VS 2.2 %, $P=0.0000$). In spite of a comparatively lower clinical pregnancy rate and implantation rate in abnormal oocytes group (26.7 % and 16.7 %), there was no significant difference as compared with normal oocytes group (50.7 % and 29.0 %) ($P>0.05$) (Table 3). When traditional ICSI was performed on dysmorphic oocytes, higher fertilization rate was achieved in comparison with rescue ICSI. This may be traced back to that traditional ICSI was performed at proper time. There was no clear blastomere interval in embryos from abnormal oocytes compared with embryos from normal oocytes, so the

evaluation of the embryos was usually a difficult decision. The more blastomere developed, the more difficult to differentiate the blastomere interval. As a result, some D3 embryos were underestimated.

The fertilization and pregnancy results in those 42 cycles stimulated with other ovarian stimulation protocols were similar to the 63 cycles as listed in this study. 2PN rate were 46.7 %, 65.5 %, 67.8 % in three different fertilization protocols. Nine conventional IVF cycles resulted in 2 embryo transfer cycles and pregnancy failure. Ten rescue ICSI cycles resulted in 6 embryo transfer cycles and 2 clinical pregnancies (33.3 %). Twenty-three traditional ICSI cycles resulted in 17 embryo transfer cycles and 7 clinical pregnancies (41.2 %). The implantation rate was 0, 13.3 % and 32.4 % respectively.

Delivery outcome

In 63 cycles with abnormal oocytes, 7 clinical pregnancies were obtained from 20 cycles of embryo transfer in rescue ICSI cycles. Six healthy babies were born from 5 full term deliveries. Another two single pregnancies aborted in the early pregnancy. In traditional ICSI cycles for abnormal oocytes group, 5 healthy babies were delivered from 4 full term pregnancies after 15 cycles of fresh embryo transfer. One single baby was aborted at mid-term pregnancy. For normal oocytes group, the miscarriage rate during clinical pregnancy was 12.5 % (27/216), and 241 babies were born from 189 deliveries.

Discussion

This study reported the lab data and clinical results in the women with severe abnormal oocytes during IVF/ICSI treatment. It was the first report about the oocytes with such severe

Table 3 Clinical and lab data for abnormal oocytes group and normal oocytes group in traditional ICSI cycles

Variable	Abnormal oocytes group($n=23$)	Normal oocytes group($n=92$)
Average ages(year)of the patients	30.5±3.9	31.6±3.2
Average infertility duration(years)	6.2±3.0 ^a	3.9±2.4
E2 level on the day of HCG	4123.8±2603.6	4260.2±2019.7
Number of oocytes retrieved	11.6±6.8	12.0±6.5
MIIOocyte rate(n)	70.3 % (187/266) ^d	85.9 % (955/1112)
Fertilization rate(n)	80.7 % (151/187)	86.6 % (827/955)
2PN rate(n)	75.9 % (142/187)	77.4 % (739/955)
High-quality embryo rate(n)	31.0 % (44/142) ^d	50.6 % (374/739)
Abandoned transfer rate per retrieval cycles(n)	30.4 % (7/23) ^d	2.2 % (2/92)
Clinical pregnancy rate per transfer cycles (n)	26.7 % (4/15)	50.7 % (38/75)
Implantation rate per transfer cycles (n)	16.7 % (6/36)	29.0 % (49/169)

Variables were compared between abnormal oocytes group and normal oocytes

^a $P<0.05$

^b $P<0.01$

^c $P<0.001$

^d $P=0.0000$

P -value <0.05 was considered statistically significant

dysmorphisms as NPVS/HZP. These two dysmorphic phenomena were always interlocking in all of the abnormal oocytes. The poor fertilization outcome of the abnormal oocytes was the main reason for some infertile women. According to the data in this study, ICSI may be the only way to obtain pregnancy for these women. In spite of the poor laboratory data developed from the abnormal oocytes, part of these women could deliver healthy babies with ART aid.

Specific oocytes dysmorphism related to fertilization protocol has not been reported before. The available literature does not discuss relations between the morphology of ZP and PVS and fertilization protocol except for some molecular researches on sperm binding related ZP glycoproteins [29–31]. In this study, women with NPVS/HZP oocytes could not obtain pregnancy after conventional IVF treatment. Most of these women had to take several IVF/ICSI cycles with repeated consistent oocyte dysmorphism and repeated implantation failure. In the IVF cycles for abnormal oocytes group, early rescue ICSI was performed with great proportion and it increased the embryo transfer rate greatly. Though normal fertilization and high-quality embryos could be got in conventional IVF cycles for abnormal oocytes, no pregnancy was achieved. The reason for that may be traced back to the D2 embryo evaluation for the limited abnormal embryos in the conventional IVF group. Many 2PN zygotes in conventional IVF cycles couldn't cleavage normally, and couldn't get normal developmental potential.

It's interesting that rescue ICSI was used effectively in the dysmorphic groups and got normal embryos development in spite of lower high-quality embryos rate. The clinical pregnancy rate and implantation rate for rescue ICSI were not different significantly in the abnormal versus normal group. From this study, it could be concluded that when the whole IVF/ICSI cycles were full of NPVS/HZP oocytes, ICSI may be the only way to get more available embryos for a better clinical result. Traditional ICSI and rescue ICSI were the ways to obtain babies delivery. Data from other 42 abnormal oocytes cycles stimulated with other COH protocols showed higher clinical pregnancy rate and implantation rate (41.2 % and 32.4 %), which may be due to more ICSI cycles had been carried out (23/42). More than that, higher primary infertility rate and longer infertility duration for the women in abnormal oocytes groups might infer the difficulty of pregnancy through spontaneous conceiving intercourse. Since this kind of oocyte might be predicted by the abnormal OCCC with sparse granular and cumulus cells around, the insemination protocol may be considered on the time of oocytes retrieval. Timely ICSI would be better for higher fertilization rate and high-quality embryo rate.

When ICSI was performed on these abnormal oocytes, the ZP and cytoplasmic membrane was punctured too easily to form a funnel. Normal fertilization could be obtained without

too much oocytes damage. So, these oocytes were different from those oocytes reported by Palermo and Nagy, which resulted in a higher rate of oocyte damage after ICSI and the ability to fertilize was remarkably reduced [32].

Recently, a study concerning the relationship between immature oocytes quantity and oocytes maturity rate found that few retrieved immature oocytes meant high fertilization rates and wide PVS incidence without correlation with the pregnancy [33]. According to the previous data reported, the oocytes with narrow PVS in our study may be related to lower maturity in vivo and not fully developed PVS. This deduction could be certified by lower MIloocyte rate in both conventional IVF cycles and traditional ICSI cycles. However, repeated NPVS/HZP oocytes retrieved in different COH cycles for these women could not elucidate it well. According to literature, enlarged PVS was negative for oocytes developmental potential. Large PVS in the injected oocytes significantly affected fertilization rate and pronuclear morphology, but had no further effect on embryo quality [7]. A Computer-assisted oocyte morphometry evaluation showed that the best quality zygotes identified by a morphological score resulted from oocytes with larger diameter and narrow perivitelline space (5.3 vs 7.1 μm ; $P<0.01$) [8], but it was different from the abnormal oocytes in our study. The extremely narrow PVS of the abnormal oocyte in our study was too narrow to be differentiated and calculated (Fig. 1c).

The ZP of the abnormal oocytes in this study was heterogeneous ZP. Aberrant ZP composition or ultrastructure could result in fertilization failure due to ineffective spermatozoon-oocyte fusion [20]. To clarify the birefringent structures of human ZP, polarized light microscopy was usually used [18, 22]. Proper thickness of the inner zona layer showed better blastulation rate [19]. As these facts had been elucidated, the ZP should have proper thickness and morphology to ensure oocytes fertilization and embryo development. The heterogeneous ZP in this study was different from the normal ZP under light inverted microscopy, which may be the reason for lower fertilization rate and reduced high-quality embryo rate.

Morphologic variations of the oocyte observed in IVF cycles may result from intrinsic factors, such as age and genetic defects, or extrinsic factors, such as COH protocol and ovarian response to COH. The occurrence of specific cytoplasmic dysmorphic phenotypes in oocytes has been suggested to reflect intrinsic defects that may negatively influence oocyte competence [11, 34, 35]. The NPVS/HZP oocytes collected in this study were specific abnormal oocytes found in our IVF lab, which maybe originated from oocytes development defects. The bi-directional communication between the oocyte and the surrounding cells is crucial for the acquisition of oocyte competence. It

is interesting that the narrow PVS and heterogeneous ZP are interlocking in all of abnormal oocytes. More than that, the abnormal OCCC might mean inadequate communication between the oocytes and granular cells (Fig. 1a). As a result, NPVS/HZP oocytes were obtained, accompanied with those abnormal characteristics.

Though no pregnancy resulted from abnormal oocytes in conventional IVF cycles, a certain normal fertilization rate (58.8 %) and high-quality embryos rate were got (35 %). Only 11 conventional IVF cycles were used for abnormal oocytes appears too small a number to draw a conclusion. Three cycles embryo transfer may not enough for a rational conclusion for these abnormal oocytes. More cases should be collected for a more convective answer. Another question was the MII oocytes rate. For the polar body was usually difficult to differentiate, the MII oocytes rate may be not conclusive. How to identify the oocytes maturation stage without considering the polar body? What would happen if all of the oocytes without clear polar bodies were given ICSI? All these questions need to be answered in the following study.

There was no improvement in the oocytes dysmorphisms till now in spite of different ovulation protocols being tried. The fact is that these oocyte dysmorphisms and bad fertilization results were the main reason for one kind of infertility. For the repeated oocytes dysmorphisms and bad clinical results, quite a few women had to accept disappointed results. It's fortunate that ICSI could overcome fertilization failure and help some women deliver their own babies. However, what confused us was that a few women with such abnormal oocytes were secondary infertility. Could those women get pregnancy through spontaneous conceiving intercourse? What's the truth of fertilization ability for this kind of abnormal oocytes? It's difficult to obtain fertilization data from healthy women, so in-depth study around the data from the infertile women would be instructive. We have no idea about the development mechanism of this kind of oocytes, but the characteristic of NPVS/HZP supplied some cues. Further study around oocytes physiology or metabolism should be carried out. Even more, understanding of oocytes genetic defects may help us to elucidate it in the near future.

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