J Assist Reprod Genet (2012) 29:905–910
DOI 10.1007/s10815-012-9823-x

ASSISTED REPRODUCTION TECHNOLOGIES

Outcome of conventional IVF and ICSI on sibling oocytes in the case of isolated teratozoospermia

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Received: 4 April 2012 / Accepted: 14 June 2012 / Published online: 26 June 2012

Abstract
Purpose To reevaluate the effect of isolated teratozoospermia on IVF and determine if there was any therapeutic benefit to isolated teratozoospermia by ICSI, since there are no widely accepted criteria for the treatment technique about isolated teratozoospermia.
Methods A total of 441 couples with >20 million and progressive motility >30 % sperm undergoing their first IVF/ICSI cycle were included in the study between 2008 and 2010, for whom at least 8 oocytes were retrived. Isolated teratozoospermia was diagnosed in 183 of the included couples, and the rest couples (normal sperm morphology) were studied as control. Sibling oocytes were randomized to be inseminated either by ICSI or IVF. Fertilization rate, embryo quality, pregnancy rate, implantation rate and spontaneous abortion rate were assessed.
Results There was no difference in the percentage of eggs fertilized, implantation rate, pregnancy rate and spontaneous abortion rate between conventional IVF and ICSI regardless of the percentage of normal morphology. The day 3 embryonic morphology and rate of development were not different despite the insemination method and percentage of normal morphology.
Conclusion Because isolated teratozoospermia did not influence the major indices of IVF and the unnecessary use of ICSI is time-consuming, costly and potential risks, couples with isolated teratozoospermia need not be subjected to ICSI.

Capsule Retrospective analysis of outcome of conventional IVF and ICSI on sibling oocytes found that in couples with isolated teratozoospermia, there may be no therapeutic benefit to ICSI.

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Keywords In vitro fertilization(IVF) · Intracytoplasmic sperm injection(ICSI) · Teratozoospermia · Sperm morphology

Introduction

In assisted reproduction techniques, decisions concerning the treatment technique (conventional IVF or ICSI) are sometimes difficult to make. There are no widely accepted criteria for the treatment technique about isolated teratozoospermia, so decisions for couples with isolated teratozoospermia are often empirical and may lead to complete fertilization failure after conventional IVF, or to the unnecessary use of ICSI. Isolated teratozoospermia is usually defined as ≤4 % normal sperm morphology at semen analysis with normal sperm count and normal progressive motility. It has been shown in many studies that semen samples with teratozoospermia produce lower fertilization rates when conventional IVF was used [1–3]. On the other hand, it was also showed in some studies that patients with teratozoospermia achieved good fertilization in conventional IVF as long as the sperm concentration and motility were within the normal range according to WHO standards [4–6]. In the series of studies about teratozoospermia, the indices of conventional IVF and ICSI were from different individuals, which may not have been equivalent because of unmeasured differences or selection bias, thus limiting the ability to compare them to some extent. Moreover, very few studies observed the difference in embryo quality and spontaneous abortion of patients with teratozoospermia between conventional IVF and ICSI.

With the development of the ovarian stimulation, semen preparation, oocyte and embryo manipulation, and embryo culture in vitro, the pregnancy rate of IVF has been increased steadily. In addition, the unnecessary use of ICSI is time-

consuming, costly, and potential risks. Although ICSI is successful, this technique is still undergoing safety evaluation. In fact, 5–10 % of oocytes may be damaged after rupture of the oolemma. The risks of disturbing the spindle during introduction of the pipette [7], the lower survival [8] and implantation rates of frozen-thawed embryos originating from ICSI than of embryos obtained by IVF [9], the malformations and chromosome abnormalities observed in the fetus [10] and the risk of transmission of infertility and other genetic defects to the offspring are still open to debate [11]. In recent 2 years, there were very few articles about the impact of isolated teratozoospermia on IVF. Therefore, with the development of assisted reproduction technique, especially the development of culture system of gamete in vitro, it was necessary to reevaluate the affect of isolated teratozoospermia on IVF and determine if there was any therapeutic benefit to isolated teratozoospermia by ICSI.

To assess the benefit of ICSI in couples with isolated teratozoospermia more carefully, we do a study on sibling oocytes from newly registered, first cycle in vitro fertilization patients with isolated teratozoospermia as the only male factor or normal sperm morphology. We attempted to correlate sperm morphology with the rates of fertilization, embryo quality, pregnancy and spontaneous abortion rate, both for conventional IVF and ICSI .

Materials and methods

A total of 441 couples undergoing their first IVF/ICSI cycle were included in the study between 2008 and 2010, for whom at least 8 oocytes were retrived. The ethical board at the West China Second Hospital of Sichuan University approved the research. All couples had purely male infertility(isolated teratozoospermia) or unexplained infertility. All couples were counseled that both ICSI and conventional IVF would be performed on the sibling oocytes simultaneously (half of the oocytes were fertilized with conventional IVF while the other half underwent ICSI), due to the possibility of total fertilization failure. Informed consent was obtained from all couples included in the study. The duration of infertility was 5.8±2.9 (mean ± SD) years. Male exclusion criteria were sperm count < 20×10^6/ml or progressive motility <30 %. All couples with 0 % strict morphology (e.g. complete teratospermia, globozoospermia) were subjected to ICSI, and excluded from the study.

The 441 cycles were divided into the following two groups: group 1 (n=258): normal sperm morphology (>4 % normal sperm morphology with >20 million and progressive motility >30 % sperm); group 2 (n=183): isolated teratozoospermia (≤4 % normal sperm morphology with >20 million and progressive motility >30 % sperm). Half of the oocytes were fertilized with conventional IVF while the other half underwent ICSI in each group.

Semen samples were collected by masturbation 3–6 days after the last ejaculation on the days of semen analysis and egg retrieval for IVF. Semen analysis was performed at our hospital within 6 months of IVF date. In both procedures semen volume, concentration and motility were evaluated according to the recommendations of World Health Organization criteria [12], and sperm morphology was assessed using the Tygerberg Strict Criteria as outlined by the World Health Organization [12] in 1999. For the morphological evaluation, 400 spermatozoa per slide were evaluated, screening at least five different areas on the slide to ensure a random distribution. The number of abnormalities in 400 spermatozoa was recorded in all semen sample. In the semen samples of the severest teratozoospermia in our study, the percentage of normal sperm morphology was 0.25 %.

The sperm samples were prepared mainly by a conventional Percoll gradients and swim-up procedure.

The female partners were down-regulated with a gonadotrophin-releasing hormone analogue, and thereafter stimulated with daily i.m. injections of human r-FSH, followed by human chorionic gonadotrophin (10 000 IU i.m.). Approximately 36 h later, the oocytes were aspirated using transvaginal ultrasound-guided retrieval. After completion of oocyte retrieval, the first cumulus–oocyte complex (COC) retrieved was allocated either to ICSI or to IVF according to blocked randomization, and the rest of the COC were allocated alternately to IVF or ICSI i.e. one to ICSI, then one to IVF, and so on [13]. Gamete manipulation for conventional IVF and ICSI was performed as the manual of IVF in our lab.

Oocytes were checked for evidence of fertilization 16–18 h after insemination. Embryo morphology was noted prior to embryo transfer which was performed 72 h after insemination of oocytes. Embryos were classified according to a simplified system based on morphological criteria: (i)Grade I embryos had equal-sized blastomeres and anucleate fragments, if present, accounted for <10 % of the volume of the embryos; (ii) Grade II embryos had blastomeres unequal in size and/or 10–30 % fragmentation; (iii) Grade III embryos had 31–50 % fragmentation; (iiii) Grade IV embryos had >50 % fragmentation; Two or three embryos were transferred depending on their morphological quality. Whenever embryos were obtained from both IVF and ICSI cycles, only the best embryos from both IVF and ICSI were chosen and a mixed ET of both IVF/ICSI embryos was performed. Excess embryos with <30 % fragmentation were frozen. Pregnancy testing was done 14 days after the embryo transfer. Clinical pregnancy was confirmed by the presence of a fetal heart on ultrasound at 6–8 weeks of pregnancy.

χ^2-test and t-test were used as appropriate; $P<0.05$ was considered statistically significant.

A power calculation with an α error of 5 % and a β error of 20 % was performed. On the basis of our previous experience and the background literature, it was assumed

that given a fertilization rate of 50 % in the IVF group and 65 % in the ICSI group [14], then a sample size of ≥134 per group was required.

Results

Comparison outcome of >4 % and ≤4 % normal spermatozoa

Female age, male age, sperm count, motile spermatozoa, mean number of oocytes/cycle, and mean number of embryos transferred were not significantly different between >4 % and ≤4 % normal spermatozoa groups (Table 1).

Because the cumulus cells around the eggs often made it difficult to determine the oocytes' maturity before conventional insemination, the fertilization rates for ICSI were not calculated per mature eggs injected but rather using the total number of oocytes(mature and immature) retrieved, and the fertilization rate for conventional IVF was also calculated using the total number of oocytes(mature and immature) retrieved.

As shown in Table 1, fertilization rate, percentage of good quality embryos of fertilized oocytes and mean number of blastomeres on day 3 were not affected by the morphology of sperm regardless of the insemination method.

Table 1 also shows outcomes in >4 % and ≤4 % normal spermatozoa groups: the implantation rate, clinical pregnancy rate and spontaneous abortion rate per pregnancy were not significantly different(P >0.05), although the latter was higher in the ≤4 % normal spermatozoa group.

Comparison outcome of different ET methods

For the >4 % normal spermatozoa group as a whole, the mean number of embryos transferred per cycle was 2.2 (Table 2). The mean number of conventional IVF embryos transferred per cycle was 2.1, the mean number of ICSI embryos transferred per cycle was 2.3, and the mean number of mixed conventional IVF and ICSI embryos transferred per cycle was 2.4.

For the ≤4 % normal spermatozoa group as a whole, the mean number of embryos transferred per cycle was 2.3 (Table 2). The mean number of conventional IVF embryos transferred per cycle was 2.4, the mean number of ICSI embryos transferred per cycle was 2.1, and the mean number of mixed conventional IVF and ICSI embryos transferred per cycle was 2.3.

The percentage of pregnancy per transfer and the implantation rate were comparable in the eight groups(group E~group L), and the differences did not attain statistical significance.

Comparison outcome by subdivision of severe teratozoospermia group

The population was subdivided into groups of >4 %, 3 %, 2 %, 1 % and ≤1 % normal morphology in Table 3. There

Table 1 Results of conventional IVF and ICSI sibling oocytes

	>4 % normal spermatozoa		≤4 % normal spermatozoa		*p*
	Conventional IVF (A group)	ICSI (B group)	Conventional IVF (C group)	ICSI (D group)	
Number of cycles	258		183		
Mean female age (years)	32.1±4.1		32.7±3.3		NS
Mean male age	35.3±3.6		34.8±4.4		NS
Sperm count(X106/ml)	49.1±27.4		43.9±25.6		NS
Motile spermatozoa (%)	38.6±12.1		40.1±14.6		NS
Mean no. oocytes/cycle	11.6±4.1		12.2±3.8		NS
Eggs allocated to IVF/ICSI(per patient)	6.4±2.7	6.1±3.2	6.5±3.7	5.9±3.6	NS
Eggs fertilized(%)	61	65	60	63	NS
Number of embryos	965	970	671	645	NS
Good quality embryos of fertilized(%)	59.1	61.5	58.5	62.0	NS
Mean no. of blastomeres on day 3	6.15	6.56	6.20	6.69	NS
Mean no. of embryos transferred	2.23±0.43		2.31±0.47		NS
Implantation rate (%)	26 %		24 %		NS
Clinical pregnancy rate	43 %		40 %		NS
Spontaneous abortion rate/pregnancy (%)	11 %		16 %		NS

NS:$p > 0.05$

Table 2 Results of different ET methods

	>4 % normal spermatozoa				≤4 % normal spermatozoa				*p*
	Conventional IVF E group	ICSI F group	Mix G group	Total H group	Conventional IVF I group	ICSI J group	Mix K group	Total L group	
No. of embryo transfers	122	54	48	214	69	44	48	161	
Embryos transferred/cycle	2.1	2.3	2.4	2.2	2.4	2.1	2.3	2.3	NS
Pregnancy per transfer(%)	42	45	44	43	40	39	43	41	NS
Implantation rate (%)	25	28	27	26	23	22	26	24	NS

The mean number of embryos transferred per cycle, the percentage of pregnancy per transfer and the implantation rate were comparable in the eight groups(group E~group L), and the differences did not attain statistical significance

were also no differences in the percentage of eggs fertilized, implantation rate and pregnancy rate per transfer. Table 3 also showed that there were no significant differences in the percentage of good quality embryos of fertilized oocytes, rate of ≥7 cells embryos of fertilized oocytes and mean number of blastomeres on day 3, despite the insemination method and percentage of normal morphology.

Discussion

In the present study we have randomly inseminated the sibling oocytes in couples with teratozoospermia or normal spermatozoa, whose husband had normal sperm count and progressive motility, either by conventional IVF or ICSI. We found that: (i) there were no differences in the percentage of eggs fertilized, implantation rate, pregnancy rate, quality of embryos and spontaneous abortion rate between conventional IVF and ICSI despite the percentage of normal morphology; (ii) in different embryo transfer methods, the percentage of pregnancy per transfer and the implantation rate were comparable, and the differences did not attain statistical significance.

A direct correlation between abnormal sperm morphology and sperm function was difficult to establish. The impact of overall sperm morphology assessment on IVF outcomes, especially in ICSI cases, has been the focus of many idiopathic infertility studies but remains controversial. On one hand, isolated teratozoospermia has a deleterious effect on both fertilization rate and pregnancy rate per cycle after IVF–embryo transfer in many previous reports [1–3]. A meta-analysis studying outcomes with conventional IVF strongly supported the diagnostic value of critical morphology assessment in sperm specimens [15]. On the other hand, some reports contradict these finding and claim abnormal sperm morphology is a poor predictor of cycle outcomes [4–6, 16, 17]. Our study showed that morphology may be not the most important sperm parameter for predicting fertilization success in IVF. When initiating IVF treatment, morphology is not critical for clinical decisions. These results challenged previous dogma that morphology was the most important sperm parameter for predicting fertilization success. We believe one reason for this is the continuous improvement in IVF techniques, especially the improvements in the standardization of media and materials and the acquisition of experience in gamete handling and embryo culture.

There were still different viewpoints about embryo quality from teratozoospermic couples. It was reported that

Table 3 Results by subdivision of severe teratozoospermia group

	>4 %		3 %		2 %		1 %		≤1 %	
	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI	IVF	ICSI
% of Eggs fertilized	61	65	56	60	63	61	60	64	61	66
Good quality embryos of fertilized(%)	59.1	61.5	61.3	65.1	56.6	65.2	59.2	63.3	57.3	62.4
Rate of ≥7 cells embryos of fertilized(%)	56.1	54.3	51.7	53.9	54.2	55.4	53.8	57.5	52.9	56.3
Mean number of blastomeres	6.15	6.56	6.12	6.53	6.21	6.71	6.58	6.45	6.19	6.72
Implantation rate (%)	26		22		24		24		25	
pregnancy rate/transfer	43		38		40		40		41	

There were no differences in the percentage of eggs fertilized,percentage of good quality embryos of fertilized, implantation rate and pregnancy rate per transfer

implantation rate, clinical pregnancy rate in ICSI-generated embryos were higher in the normal sperm group compared to the teratozoospermic group [2]. In conventional IVF, poor sperm morphology resulted in poor embryo quality [3, 18]. On the other hand, Terriou et al. compared the embryo quality in 102 cycles of IVF-ET using normal frozen donor semen and 94 cycles of IVF –ET using husbands' teratozoospermic sperm, and found that teratozoospermia didn't influence embryo quality [19]. Cohen et al. [20] reported that in ICSI there was no significant correlation between the percentage of normal sperm forms, and rates of implantation and fertilization. Moreover, it was showed that there was no significant difference in fertilization and pregnancy rates after ICSI in patients with severe teratozoospermia as compared with less severe forms [21]. In our study, there was no difference in implantation rate, pregnancy rate, percentage of good quality embryos of fertilized oocytes and spontaneous abortion rate between conventional IVF and ICSI regardless of the percentage of normal morphology. We also found that in different embryo transfer methods, the percentage of pregnancy per transfer and the implantation rate were comparable. Furthermore, the quality of embryos obtained in terms of day 3 embryonic morphology and rate of cellular development were comparable, irrespective the insemination method and percentage of normal morphology. The oocyte factors that affect early embryo development were similar in conventional IVF and ICSI groups in our study. The embryonic genome was not activated before day 3 [22]. It was suggested that the embryo may be able to repair low levels of DNA damage introduced by the sperm nucleus [23, 24]. So we speculated that the morphological appearance of the spermatozoa may not correlate to inherent quality, and that once fertilization is attained with such spermatozoa, it does not descend the embryos' development potential. This was in accordance with the conclusion of a recent meta-analysis, which was that isolated teratozoospermia was not associated with a statistically significantly decreased probability of pregnancy with assisted reproduction [25].

We acknowledge that there may be several potential limitations when evaluating our study. Firstly, our study is retrospective, and there may be possibility to introduce selection bias. However, there were a large number of couples that were enrolled in the protocols by several physicians. Attempting to minimize the difference among groups of patients, we conducted the study comparing conventional IVF and ICSI in sibling oocytes. To maximize the validity of our study, strict selection and analysis criteria were established. Couples undergoing their first IVF/ICSI cycle and for whom at least 8 oocytes were retrived were included in our study. Oocytes were randomly allocated into conventional IVF and ICSI groups. Thus, bias from a single physician and from the inequality between the groups was limited. Secondly, it is also important to note that, whether it is possible that the criterion for defining teratozoospermia was not stringent enough or too stringent. Semen analysis of every couple was performed by two different technologists, both of whom has been professionally trained and has performed semen analysis for several years in our university IVF center. The sperm morphology was verified by averaging the results from the two technologists. In summary, many potential sources for bias have been addressed and avoided as far as possible, allowing clinical application of the results practically.

In conclusion, couples with isolated teratozoospermia need not be subjected to ICSI, because isolated teratozoospermia did not influence the major indices of IVF, and ICSI adds extra time for the embryologist and extra expense for the infertile couple.

Acknowledgements The authors wish to thank the staff of the reproductive center of the West China Second University Hospital for their contribution.

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