J Assist Reprod Genet (2017) 34:1179–1183
DOI 10.1007/s10815-017-0966-7

ASSISTED REPRODUCTION TECHNOLOGIES

Pre-implantation genetic diagnosis—should we use ICSI for all?

Baruch Feldman[1,2,3] · **Adva Aizer**[1] · **Masha Brengauz**[1] · **Keren Dotan**[2] · **Jacob Levron**[1,3] · **Eyal Schiff**[1,3] · **Raoul Orvieto**[1,3,4]

Received: 14 March 2017 / Accepted: 31 May 2017 / Published online: 13 June 2017

Abstract
Objective Intracytoplasmic sperm injection (ICSI) is commonly used during pre-implantation genetic diagnosis (PGD) in vitro fertilization (IVF), aiming to eliminate the risk of contamination from extraneous sperm DNA. Recently, ICSI "overuse" in non-male infertility has been doubted, since it does not offer an advantage over IVF. Prompted by the aforementioned observations, we sought to assess the accuracy of IVF vs ICSI in PGD cases, as might be reflected by a difference in the prevalence of discarded embryos as a consequent of parental contamination.
Methods Cohort-historical study of all consecutive patients admitted to the IVF-PGD program in a large tertiary center. The percentages of complete, incomplete diagnosis, PCR failure, abnormal embryos, and the contamination rate with paternal DNA in the IVF-only and the ICSI-only groups. We reviewed the computerized files of all consecutive women admitted to our IVF for a PGD-PCR cycle. Patients were divided accordingly into three groups: an IVF group—where all the oocytes underwent IVF only, an ICSI group—where all oocytes underwent ICSI, and a mixed group—where sibling oocytes underwent both IVF and ICSI. The laboratory data and the genetic diagnostic results were collected and compared between the different insemination groups.
Results Nine-hundred and twenty-seven patients underwent IVF-PGD cycles in our program, 315 in the IVF group, 565 in the ICSI group, and 47 in the mixed group. No differences were observed in fertilization rates, the percentage of embryos available for biopsy, and the percentages of complete, incomplete diagnosis, PCR failure, or abnormal embryos, between the IVF-only and the ICSI-only groups and between the IVF and the ICSI of sibling oocytes in the mixed group. Moreover, contamination with paternal DNA, through contamination with sperm cells, was negligible. Not one single case of misdiagnosis was encountered during the study period.
Conclusion It might be therefore concluded that IVF should be the preferred insemination methods in PGD cycles, and ICSI should be indicated only in cases of male-factor infertility.

Keywords PGD · PCR · ICSI · IVF · Contamination

Introduction

Pre-implantation genetic diagnosis (PGD) allows patients who are carriers of single-gene disorders or carriers of structural chromosome abnormalities to select unaffected embryos for transfer, and reduce the transmission of genetic disorders to the offspring.

With the current advent of new cytogenetic techniques, the procedures employed for PGD are rapidly evolving with the consequent improve in their utilization. Moreover, PGD, based on single embryonic cell testing, has already been applied in a wide range of genetic diseases and chromosomal

✉ Raoul Orvieto
raoul.orvieto@sheba.health.gov.il

[1] Infertility and IVF Unit, Department of Obstetrics and Gynecology, Sheba Medical Center, Ramat-Gan, Israel

[2] Danek Gertner Institute of Human Genetics, Sheba Medical Center, Ramat-Gan, Israel

[3] Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

[4] The Tarnesby-Tarnowski Chair for Family Planning and Fertility Regulation, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel

aberrations. PGD-polymerase chain reaction (PCR) protocols are most frequently used to amplify the small DNA content achieved from the blastomere biopsy, with the consequent high risk of contamination.

It was therefore suggested that denuding the oocyte of cumulus cells followed by intracytoplasmic sperm injection (ICSI) instead of insemination/in vitro fertilization (IVF) might significantly reduce the risk of contamination from extraneous sperm attached to the zona pellucida or non-decondensed sperm within blastomeres or cumulus cell [1, 2].

According to the ESHRE PGD Consortium data collection XIII on PGD cycles for monogenic diseases, ICSI was used in the majority of cycles [99% of cycles to oocyte retrieval (OR)], with day 3 cleavage-stage embryo biopsy most frequently used (93% of cycles to PGD), and PCR was still the most widely used first-line method of DNA amplification (90% of cycles to PGD) [3].

Intracytoplasmic sperm injection was developed for the treatment of couples with severe male-factor infertility to ensure high fertilization and pregnancy rates regardless of semen characteristics. Since its introduction [4], ICSI has gained tremendous popularity in assisted reproductive technology (ART) units throughout the world and has become a routine procedure in many of them. However, its "overuse" in non-male infertility has been recently doubted [5], since it does not offer an advantage over IVF [6, 7] nor improves clinical outcomes [8].

In our PGD program, we have experienced three different periods. At the beginning, we used solely ICSI for all PGD oocytes; later, we used ICSI only in male-factor infertility, and during the last 2 years, in patients with unexplained infertility undergoing their first IVF-PGD attempt, we offer the use of IVF and are subjecting some sibling oocytes to ICSI, in order to avoid total fertilization failure.

Prompted by the aforementioned observations, we sought to assess the accuracy of IVF vs ICSI in PGD cases, as might be reflected by a difference in the prevalence of discarded embryos as a consequent of parental contamination.

Patients and methods

Patients

We reviewed the computerized files of all consecutive patients admitted to our IVF-PGD program from January 2006 to December 2014. Only patients undergoing PGD for the prevention of single-gene disorders, based on multiplex PCR programs designed for haplotyping using informative microsatellite markers, were included. The elimination of bias in this selection, for the purposes of this study, was achieved by including only patients who reached the ovum pickup (OPU) stage and had at least one oocyte available for fertilization.

All the usual indications for IVF/ICSI and accepted protocols for controlled ovarian hyperstimulations (COHs) described in [9] were included. The selection of type of COH protocol used was the decision of the treating physician and largely dependent on the fashion at the time. In all protocols, gonadotropins were administered in variable doses, depending on patient age and/or ovarian responsiveness in previous cycles, and further adjusted according to serum E2 levels and vaginal ultrasound measurements of follicular diameter obtained every 2 or 3 days. The criteria for hCG administration were at least three follicles with the diameter of 18 mm with appropriate peripheral E2 levels. Oocytes were retrieved 36 h after hCG administration.

Laboratory procedure

On the day of oocyte retrieval, semen samples were obtained by masturbation, collected in sterile tubes, and delivered to the IVF laboratory within 60 min.

Sperm parameters were evaluated according to the World Health Organization (WHO) criteria [10]. The semen processing was carried by commercially available density gradients (80/40, SAGE, USA), followed by centrifugation at 800×*g* for 20 min. The gradient was removed, keeping the pellet undisturbed. The pellet was washed once for ICSI or twice for insemination *f* (800×*g*, 5 min) in 1 ml of pre-warmed gamete medium (COOK Medical, USA), and the final pellet was overlaid with 100–300 μl of the Gamete medium, followed by incubation at room temperature for 30 min. The supernatant was then removed and assessed for sperm concentration.

The morning after IVF/ICSI fertilization, oocytes were washed twice and transferred into 6-day growth media (1-STEP SAGE USA/GLOBAL TOTAL, LIFE GLOBAL USA), followed by assessing fertilization using ×200 inverted microscope. The zygotes were then cultured and reevaluated on days 2 and 3 for embryo development.

On day 3, embryos underwent blastomere biopsy using a micromanipulation system (Narashige, Japan) fitted on an inverted microscope (Diaphot 300, Nikon, Japan). A laser system (ZILOS-tk, Hamilton Thorne) was used for dissection of the zona pellucida prior to biopsy. A single blastomere was removed from each embryo and evaluated under ×400 inverted microscope for its integrity, presence of a nucleus, and being free from other cells\debris. Each blastomere was routinely washed in three drops of clean biopsy medium, prior to its transfer to the PCR tube, ensuring a pure sample. Moreover, a sample from the last drop of the washing medium was also collected in a different PCR tube and transferred for molecular analysis as control to detect possible contamination.

IVF vs ICSI

From January 2006 to October 2009, ICSI was used exclusively for all PGD patients. Since November 2009, fertilization was achieved by IVF or ICSI, regardless of the use of PGD, and according to clinical indications (i.e., ICSI for male factor infertility and IVF in cases of unexplained infertility). In the last few years, we offered patients with unexplained infertility undergoing their first IVF-PGD attempt to use IVF but to subject some of the sibling oocytes to ICSI, in order to avoid total fertilization failure.

PGD cycles were stratified accordingly into three groups: an IVF group—in which all the oocytes underwent IVF only, an ICSI group—in which all oocytes underwent ICSI, and a mixed group—in which sibling oocytes underwent both IVF and ICSI.

Molecular diagnosis

Establishing haplotyping was a necessary step in the preparation for PGD. Several informative microsatellite markers were selected for each patient in preparation for PGD. At least two informative polymorphic short tandem repeats (STRs) were linked for each family on either side of the mutant allele. Primers suitable for multiplex PCR were carefully designed for each marker, and the diagnostic protocol was examined using DNA samples of appropriate family members who were carriers or non-carriers of the specific familial mutant allele. Each case was pre-validated in a model specifically designed for each family. Validation was achieved by employment of genomic DNA samples for haplotyping and highly diluted DNA samples for pre-PGD validation, mimicking single-cell molecular testing.

Maternal and paternal DNA samples were always included in each case; therefore, these informative markers could differentiate between maternal and paternal contribution of alleles to all normal or abnormal embryos. The genetic constitution of each normal embryo is made-up from one maternal and one paternal allele. An embryo with unequal parental contribution is easily detectable while the origin of the extra allele is straightforwardly diagnosed by the pre-validated markers. Using multiple informative markers in all cases allows us to diagnose all embryos with uniparental disomy or aneuploidy.

Embryos were usually biopsied for PGD on the morning of day 3 post-fertilization, and the blastomeres were shipped to the molecular laboratory for analysis. The pre-IVF molecular diagnostic protocol was applied as designed for each patient and results were mailed to the IVF laboratory director, usually 8 to 24 h following the time of biopsy.

Molecular diagnoses of each embryo are classified as follows:

Complete diagnosis—unaffected or affected embryo according to the genetic disorder examined
Incomplete diagnosis—suspected allele dropout (ADO) or recombination
PCR failure—no DNA is available for diagnosis
Abnormal—the embryo has abnormal assembly of alleles—i.e., any structure different from one maternal and one paternal alleles matching the known haplotype, e.g., trisomy, monosomy, or uniparental disomy (UPD).

The molecular diagnosis of each embryo was grouped according to its specific method of insemination.

Statistics

Differences in variables between the different insemination groups were statistically analyzed with chi-squared test as appropriate. A p value of <0.05 was considered significant.

IRB approval

The study was approved by the IRB of the Sheba Medical Center ethical committee (IRB approval no. 9918).

Results

Three-hundred and forty-five patients underwent 1127 cycles in our PGD program between January 2006 and December 2014. Nine-hundred and twenty-seven cycles matched the inclusion criteria and were included in the study, 315 cycles in the IVF group, 565 in the ICSI group, and 47 in the mixed IVF-ICSI group. The laboratory/embryological data are presented in Table 1. No significant differences were observed between the groups in fertilization rates and the percentage of embryos available for biopsy. The molecular results are presented in Table 2. No significant differences were observed in the percentages of complete, incomplete diagnosis, PCR failure, or abnormal embryos, between the IVF-only and the ICSI-only groups and between the IVF and the ICSI of sibling oocytes in the mixed group.

While we carefully examined the relative parental contribution of the 514 embryos diagnosed as "abnormal," i.e., trisomy and UPD cases, we found that the relative parental contributions were not different between the IVF and ICSI groups [58/196 (29.6%) and 99/318 (31.1%), in IVF and ICSI groups, respectively; $p = 0.71$]. Of notice, in order to demonstrate a difference of 5% in the percentage of paternal contribution to abnormal embryos at a power of 80% and an alpha value of 5%, 114 samples are needed in each group.

Moreover, we could not find any significant difference in contamination rates of the washing medium samples, between the IVF and ICSI study groups. Contamination with paternal

Table 1 The laboratory data and the molecular/genetic diagnostic results in the different study groups

	IVF-only group	ICSI-only group	Mixed group		Total	ESHRE consortium data collection XIII (2015)
			IVF	ICSI		
No. of cycles	315	565	47	47	927	14,968
No. of oocytes retrieved	3,136	5,739	304	322	9,501	200,404
No. of fertilized oocytes (%)	2,184 (69.6%)	3,379 (58.8%)	195 (64.1%)	225 (69.8%)	5,983 (62.9%)	123,022 (61.4%)
No. of embryos Bx (percent of fertilized oocytes)	1,839 (84.2%)	2,918 (86.3%)	163 (83.6%)	173 (76.9%)	5,093 (85.1%)	91,710 (75.5%)
Tested embryos						
Complete (%)	1,314 (72.9%)	2,066 (70.8%)	125 (76.7%)	124 (71.7%)	3,656 (71.8%)	
Unaffected	717 (38.9%)	1,056 (36.2%)	68 (41.7%)	61 (35.2%)	1,902 (37.3%)	30,699
Incomplete (%)	86 (4.7%)	161 (5.5%)	5 (3.1%)	7 (4.0%)	259 (5.1%)	
PCR failure (%)	233 (12.7%)	390 (13.4%)	16 (9.8%)	25 (14.4%)	664 (13.0%)	
Abnormal (%)	179 (9.7%)	301 (10.3%)	17 (10.4%)	17 (9.8%)	514 (10.1%)	
P value (IVF vs ICSI)		0.38		0.56		

Complete—affected or unaffected, incomplete—suspected ADO or recombination, PCR failure—no embryonal DNA is available for PCR, abnormal—the embryo has abnormal assembly of alleles—i.e., any configuration of alleles different from one maternal and one paternal alleles, unaffected—healthy transferable embryos

DNA, through contamination with sperm cells, was negligible. Paternal alleles were detected in very few cases of the washing medium samples; the contamination in both IVF and ICSI study groups were 17/2002 (0.8%) and 22/3091 (0.7%), respectively.

Four-hundred and sixty-three ICSI and 286 IVF cycles have reached embryo transfer during the study period. Eight-hundred and fifty-seven ICSI and 479 IVF unaffected embryos were transferred, resulting in 28.0 and 27.2% pregnancy rates per transfer, respectively. Eighty-eight ICSI and 56 IVF newborns were tested pre- or post-natally. No single case of misdiagnosis was observed during the study period.

Discussion

In the present study of patients undergoing IVF treatment cycle, utilizing PGD, based on multiplex PCR programs designed for haplotyping using informative microsatellite markers, the use of IVF yields comparable results to ICSI, in terms of the embryological, genetic/molecular diagnostic outcomes and contamination rate. Not one single case of misdiagnosis was encountered during the study period.

Single-cell molecular analysis confronts major technical problems stemming from the minute initial amount of DNA and the many PCR cycles required for diagnosis. Amplification failure (AF), ADO, and unrelated DNA contamination are the most challenging. However, reports already exist in relation to the credibility and precision of these diagnostic methods [11].

Our overall performance and the outcome of IVF and ICSI cycles were comparable to the ESHRE consortium data collection XIII [3] (Table 1).The fertilization rates (69.6, 58.8, 62.9, and 61.4%) and percentages of embryos undergoing biopsy per fertilized oocyte (84.2, 86.3, 85.1, and 75.5%) were comparable between oocytes undergoing IVF, ICSI, all oocytes, and the ESHRE data, respectively.

Of the embryos undergoing biopsy, overall, 71.8% were designated to have a complete diagnosis—unaffected or affected embryo according to the genetic disorder examined, 72.9% in the IVF-only and 70.8% in the ICSI-only groups. Moreover, of the biopsied embryos, the percentages of unaffected/transferable embryos were 37.3% overall, 38.9% in the IVF-only, and 36.2% in the ICSI-only groups, figures that are in line with the ESHRE data [3], reporting that 33.5% of diagnosed embryos were genetically transferable.

Our observations report on similar results when using IVF or ICSI in case of non-male factor infertility undergoing PGD-PCR protocols, abrogating previous prejudice that ICSI is mandatory in such cases to avoid the risk of contamination from extraneous sperm attached to the zona pellucida or non-decondensed sperm within blastomeres or cumulus cell [1, 2].

Table 2 Paternal contribution of alleles to embryos with aneuploidy and uniparental disomy in IVF vs ICSI cases

	Abnormal	Non-paternal contribution	Paternal contribution	*p* value
IVF (no. of embryos)	196	138 (70.4%)	58 (29.6%)	0.71
ICSI (no. of embryos)	318	219 (69.9%)	99 (31.1%)	

We found that the relative paternal or maternal contributions to all abnormal embryos, i.e., aneuploidy and UPD cases, were not different between the IVF and ICSI groups. Moreover, contamination with paternal DNA, through contamination with sperm cells, was negligible.

Conclusion

It might be therefore concluded, that in PGD cycles, IVF should be the preferred insemination methods and ICSI should be reserved only for cases of male-factor infertility.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

All authors read and approved the final manuscript.

References

1. Harton GL, Magli MC, Lundin K, Montag M, Lemmen J, ESHRE PGD, et al. Consortium/embryology special interest group-best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). Hum Reprod. 2011;26:41–6.
2. Berger VK, Baker VL. Preimplantation diagnosis for single gene disorders. Semin Reprod Med. 2014;32:107–13.
3. De Rycke M, Belva F, Goossens V, Moutou C, SenGupta SB, ESHRE PGD, et al. Consortium data collection XIII: cycles from January to December 2010 with pregnancy follow-up to October 2011. Hum Reprod. 2015;30:1763–89.
4. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic sperm injection of single spermatozoon into the oocyte. Lancet. 1992;340:17–8.
5. Evers JLH. Santa Claus in the fertility clinic. Hum Reprod. 2016;31:1381–2.
6. Bhattacharya S, Hamilton MP, Shaaban M, Kalaf Y, Seddler M, Ghobara T, et al. Conventional in-vitro fertilization versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. Lancet. 2001;357:2075–9.
7. Tannus S, Son WY, Gilman A, Younes G, Shavit T, Dahan MH. The role of intracytoplasmic sperm injection in non-male factor infertility in advanced maternal age. Hum Reprod. 2017;32:119–24.
8. ASRM, the practice committees of the American Society for Reproductive Medicine and Society for Assisted Reproductive Technology. Intracytoplasmic sperm injection (ICSI) for non-male factor infertility: a committee opinion. Fertil Steril. 2012;98:1395–9.
9. Orvieto R, Brengauz M, Feldman BA. Novel approach to normal responder patient with repeated implantation failures—a case report. Gynecol Endocrinol. 2015;31:435–7.
10. World Health Organization. WHO Laboratory manual for the examination and processing of human semen. 5th ed. Geneva: WHO Press; 2010.
11. Dotan K, Feldman B, Goldman B, Peri Y, Peleg L. The single cell as a tool for genetic testing: credibility, precision, implication. J Assist Reprod Genet. 2010;27(6):335–41.