Original Article

To Compare Aneuploidy Rates Between ICSI and IVF Cases

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Abstract

Introduction: Intracytoplasmic sperm injection (ICSI) currently helps many couples with male infertility. However, ICSI procedure may cause asynchronous sperm decondensation. This could introduce a risk for aneuploidy. The ICSI technique also could cause damage to the second meiotic spindle during injection and cause significantly abnormal pairing of chromosomes when compared with In vitro fertilization (IVF). In this study, we have examined whether ICSI has a higher incidence of aneuploidy when compared with IVF. **Material and Methods:** A retrospective study was conducted on 36 individuals. Common numbers of chromosome abnormalities were detected using fluorescent in-situ hybridization (FISH). Seven probes were used to detect chromosome X, Y, 13, 16, 18, 21, and 22. Chi-square test was used for statistical analysis and presented as odd ratios with confidence intervals. **Results:** The age range was 26 through 44 (mean age 35.5) for IVF and 25 through 46 (mean age 35.8) for ICSI. From the 36 egg retrievals, 57 embryos were obtained from nine individuals using IVF and 183 embryos were obtained from 27 individuals using ICSI. For the IVF group, 37 of the 57 examined embryos were abnormal (65%), whereas 128 of 183 examined embryos were abnormal for the ICSI group (69.9%). Among the 57 embryos from the IVF cases, the number of absolute abnormal chromosomes were as follows: X&Y chromosomes: 4 (12.9%), chromosome 13: 9 (29%), chromosome 16: 7 (22.5%), chromosome 18: 6 (19.3%), chromosome 21: 8 (25.8%), chromosome 22: 10 (32.2%). For the ICSI embryos: X and Y chromosomes: 18 (14%), chromosome 13: 34 (26.5%), chromosome 16: 23 (18%), chromosome 18: 23 (18%), chromosome 21: 26 (20.3%), chromosome 22: 31 (24.2%). The odds ratios for the difference between IVF and ICSI for each chromosome were as follows: X&Y chromosomes: 1.53 (0.598-3.916), chromosome 13: 0.969 (0.443-2.122), chromosome 16: 0.709 (0.307-1.639), chromosome 18: 1.650 (0.650-4.188), chromosome 21: 0.777 (0.350-1.724), chromosome 22: 0.647 (0.311-1.348). Overall no significant difference between two insemination procedures was seen 0.948 (0.678-1.324). **Conclusions:** As a result; ICSI does not create a significantly higher aneuploidy number when compared with IVF as examined by FISH analysis of seven chromosome pairs.

Keywords: *Aneuploidy, infertility, ICSI, IVF, fluorescent in-situ hybridization*

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Introduction

Embryonic aneuploidies may be responsible for pregnancy failure in many IVF patients. In recent years, fluorescent in-situ hybridization (FISH) for multiple chromosomes has been used to document a high frequency of chromosomal errors and aneuploidy in human preimplantation embryos and, after embryo biopsy, to select embryos that are more likely to implant.

In both ICSI and IVF, selection of good quality embryos for transfer is based on morphological criteria. However, many women fail to achieve a pregnancy even after "good quality" embryo transfer.[1] Such morphologically normal embryos contain abnormal number of chromosomes (aneuploidies). Many reports were published that showing numerical chromosome abnormalities in morphologically normal human cleavage

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stage embryos.[2] Aneuploidic embryos is expected to not develop to term, preimplantation genetic screening (PGS) for aneuploidies was introduced to increase for pregnancy rates.[3]

ICSI procedure can be cause asynchronous sperm condensation. This could introduce as a risk for aneuploidy. The ICSI technique also could cause damage to the second meiotic spindle during injection and cause significantly abnormal pairing of chromosomes when compared with IVF. Natural selection mechanism was bypassed in ICSI and this could potentially lead to higher aneuploidy rates.[4,5]

Some studies were examined cytogenetic results following IVF and ICSI. The results from these studies were contradictory. One study was showed that there was similar for aneuploidy rates in IVF and ICSI procedure.[6] However another study found a significantly higher aneuploidy rate in ICSI.[7] The relationship between abortion, aneuploidy, and chromosomal abnormalities has long been known. In fact, it is clear that chromosomal abnormalities are the most common causes of abortions. It is conceivable to establish that there is relationship between aneuploidy and abortion. Essentially, this relationship is an indirect relationship. Currently, direct studies have been done in order to detect aneuplioidy in embryos, but there is a small number of studies about direct studies because they are very expensive and problematic in terms of ethical approval. The literature on pregnancy outcomes after ICSI is limited and inconclusive concerning the risk of miscarriage and aneuploidy. ICSI bypasses natural selection mechanisms and could potentially lead to higher first trimester aneuploidy rates. The theoretical procedure-dependent risks include (i) physical or biochemical disturbance of ooplasm or the meiotic spindle, (ii) injection of biochemical contaminants, (iii) injection of sperm-associated exogenous DNA. Procedure-independent risks include (iv) injection of sperm carrying a chromosomal anomaly, (v) transmission of genetic defect, which may be related to the underlying male factor infertility, (vi) male gamete structural defect, (vii) anomalies of sperm activating factors, (viii) potential for incorporating sperm mitochondrial DNA, and (ix) female gamete anomalies. There is still ongoing debate for concern about IVF and ICSI procedures to increase the aneuploidy rates. In this study, we have examined whether ICSI has a higher incidence of aneuploidy rate when compared with IVF.

Materials and Methods

Patients

The initial outpatient consultation consisted of combined genetic and reproductive assessment and counseling, as well as psychological advises when required. Subsequently, eligibility of the couple for Preiplantation genetic diagnosis was assessed for the indication concerned. Treatment with ovarian stimulation and IVF/ICSI plus PGS was initiated after completion of the genetic testing. This study did not include results of cycles where frozen-thawed embryos after PGS were transferred. The couples were categorized according to age, the method of pituitary suppression [gonadotropin releasing hormone (GnRH) agonist versus GnRH antagonist] and genetic categories according to availability of transferable embryos after PGS. The category of 50% genetically transferable embryos includes X-linked dominant conditions in which carrier female embryos may result in affected offspring, and hence have not been transferred. This category also includes X-linked recessive with sexing and transfer of XX embryos only. The category of other chromosomal abnormalities includes structural or numerical chromosomal abnormalities.

Ovarian stimulation and oocyte retrieval

Pituitary desensitization was carried out in an agonist protocol, using GnRH analogues (buserelin, Suprefact; Hoechst, Frankfurt, Germany), in combination with human menopausal gonadotrophins (hMG) (Menopur; Ferring Pharmaceuticals A/S, Copenhagen, Denmark), or recombinant FSH (Puregon; NV Organon, Oss, The Netherlands), or in an antagonist protocol with a GnRH antagonist (ganirelix, Orgalutran, NV Organon) combined with recombinant FSH or hMG. The starting dose of gonadotrophins was based on the female age and/or previous response to ovarian stimulation (range 75-450 IU). Human chorionic gonadotrophin (hCG) (10,000 IU, Pregnyl; NV Organon or Profasi, Serono, Geneva, Switzerland) was administered for final oocyte maturation. Transvaginal ultrasound-guided oocyte collection (OC) was scheduled 36 h after hCG administration. OC was carried out under premedication with pethidine 1 mg/kg IM and paracervical block with mepivacaine hydrochloride, or under general anesthesia when indicated.

ICSI, IVF embryo culture and biopsy

Oocyte-cumulus complexes (OCC) were identified with a dissecting microscope, and assigned alternatively in order of retrieval into two groups. For both groups, the OCC were placed in 1 ml of IVF medium (Medicult, Lyon, France) in tubes, and incubated in a humidified 37°C incubator in 5% CO_2 in air. Semen was prepared by a 45-90% discontinuous gradient method using PureSperm (Nidacon International AB, Gothenburg, Sweden). After preparation, the same semen sample was used for both conventional IVF and ICSI. All the oocytes in one group were treated by conventional IVF and were inseminated ~4 h after retrieval with 60,000 motile sperm in 1

mL of IVF medium. The other group of oocytes was treated by ICSI. Immediately before micromanipulation, cumulus and corona cells were removed enzymatically by incubating the oocytes in 1 mL of IVF medium containing 80 IU/mL hyaluronidase (Medicult) for 2-3 min. The denuded oocytes were examined to assess integrity and maturity. Only those oocytes that had extruded the first polar body (metaphase II oocytes) were microinjected. Immediately before injection, the sperm suspension was added to a 50-μL droplet of polyvinylpyrrolidone (PVP; Medicult). Oocytes were microinjected ~5 h after retrieval in microdroplets of IVF medium covered with lightweight paraffin oil. A single motile spermatozoon with apparently normal morphology was immobilized by touching its tail with the injection pipette and aspirated tail-first into the injection pipette. The sperm was microinjected into the ooplasm at the 3-o'clock position, the polar body being oriented at the 6- or 12-o'clock position.

Assessment of fertilization and embryo quality

Fertilization was assessed 15-18 h after insemination or microinjection. For conventional insemination, the cumulus and corona cells surrounding the oocytes were removed by dissection using a thin pipette in an organ culture dish. The injected oocytes were observed for any sign of damage and for the presence of pronuclei. Oocytes were classed as fertilized if two pronuclei (2PN) were present and the second polar body had been extruded. Abnormally fertilized oocytes (1PN or 3PN) were excluded. Normally fertilized oocytes were left in culture for a further 24 h. Embryos were classified according to a simplified system based on morphological criteria: (i) type A embryos had equal-sized blastomeres and anucleate fragments, if present, accounted for <10% of the volume of the embryos, (ii) type B embryos had blastomeres unequal in size and/or 10-30% fragmentation, and (iii) type C embryos had > 30% fragmentation.

In the procedure of PGS, ICSI was the method of choice rather than classical IVF to prevent contamination with residual sperm DNA and to maximize the fertilization rate in PGS in polymerase chain reaction (PCR)-based PGS.

Fertilization was assessed 16-18 hours after ICSI. Further development was evaluated in the morning of day 2 and again on day 3 when embryos were evaluated before biopsy. According to the number of anucleate fragments, the embryos were subdivided into grades A, B, C, and D as described previously. Embryo biopsy was performed on day 3 from the 5-cell stage and 6-cell stage of grades A, B, and C embryos. Laser-assisted biopsy was used to breach the zona pellucida at our center. The aspiration method was used to remove one or two blastomeres from the embryo. For PCR analysis, each blastomere was placed in a solution that lysis the cell and releases the DNA. For FISH purposes, a blastomere was spread on a slide using the HCl/ Tween 20 method.

Genetic diagnosis

The PCR procedures were performed as previously described. Multiplex PCR is the simultaneous amplification of two or more DNA sequences. It has become the standard method of DNA amplification at single cell level over the years, reducing the risk of undetected contamination and allele drop out by the using linked markers alone, or combined with the detection of a specific mutation. Numerical chromosomal analysis was performed using a FISH procedure that allows analysis of chromosomes X and Y; chromosome 18, 13 and 21 if the fluorochromes available at the time of analysis, and also chromosomes 16 and 22 in a second round of hybridization. By this approach, the embryos carrying normal or balanced chromosomes can be separated from the embryos carrying unbalanced chromosomes

Statistical analysis

Statistical analysis was performed using Statistical Program for Social Science (SPSS 13.0, Chicago, Illinois, USA) software. Continuous variables were reported as the mean ± standard deviation or as the median and range, depending on their distribution, with a normal distribution defined using the one-way ANOVA test. Categorical variables were compared using the chi-square test. The significance level for all analyses was set at $P < 0.05$. All data are reported as means with their associated standard deviations. Odds ratios and 95% confidence intervals (CIs) are shown where appropriate. The study protocol was approved by the Institutional OGA (Obstetrics and Gynecology Associates) Medical Center.

Results

The age range was 26 through 44 (mean age 35.5) for IVF and 25 through 46 (mean age 35.8) for ICSI. From the 36 egg retrievals, 57 embryos were obtained from nine individuals using IVF and 183 embryos were obtained from 27 individuals using ICSI. For the IVF group, 37 of the 57 examined embryos were abnormal (65%), whereas 128 out of 183 examined embryos were abnormal for the ICSI group (69.9%) [Table 1].

Among the 57 embryos from the IVF cases, the number of absolute abnormal chromosomes were as follows: X&Y chromosomes: 4 (12.9%), chromosome 13: 9

Table 1: Demographic characteristics of IVF and ICSI patients, duration of infertility, distributions of embryo and abnormal embryo

ART type	Number of cases	Age	Duration of Infertility	The total number of embryo	The number of abnormal embryo
IVF	9	35.5 (26–44)	4 (1–9)	57	37 (65%)
ICSI	27	35.8 (25–46)	5 (1–8)	183	128 (69.9%)

Table 2: It shows the rate of chromosomal abnormalities in IVF and ICSI cases by FISH analysis

Abnormal Chromosomes	IVF n%	ICSI n%
Chromosome XY	4 (12.9%)	18 (14%)
Chromosome 13	9 (29 %)	34 (26.5%)
Chromosome 16	7 (22.5%)	23 (18%)
Chromosome 18	6 (19.3%)	23 (18%)
Chromosome 21	8 (25.8%)	26 (20.3%)
Chromosome 22	10 (32.2 %)	31 (24.2%)
Total	44	155

Table 3: Odds ratios showing the relationship between abnormal chromosomes and IVF-ICSI

Abnormal chromosome	Odds ratio in ICSI and IVF cases
Chromosome XY	1.53 (0.598–3.916)
Chromosome 13	0.969 (0.443–2.122)
Chromosome 16	0.709 (0.307–1.639)
Chromosome 18	1.650 (0.650–4.188)
Chromosome 21	0.777 (0.350–1.724)
Chromosome 22	0.647 (0.311–1.348)
Total	0.948 (0.678–1.324)

(29%), chromosome 16: 7 (22.5%), chromosome 18: 6 (19.3%), chromosome 21: 8 (25.8%), chromosome 22: 10 (32.2%). For the ICSI embryos: X&Y chromosomes: 18 (14%), chromosome 13: 34 (26.5%), chromosome 16: 23 (18%), chromosome 18: 23 (18%), chromosome 21: 26 (20.3%), chromosome 22: 31 (24.2%) [Table 2].

The odds ratios for the difference between IVF and ICSI for each chromosome were as follows: X and Y chromosomes: 1.53 (0.598-3.916), chromosome 13: 0.969 (0.443-2.122), chromosome 16: 0.709 (0.307-1.639), chromosome 18: 1.650 (0.650-4.188), chromosome 21: 0.777 (0.350-1.724), chromosome 22: 0.647 (0.311-1.348). Overall no significant difference between two insemination procedures was seen 0.948 (0.678-1.324) [Table 3].

Discussion

Although conventional IVF pregnancy rates are similar to ICSI pregnancy rate in case of successful fertilization achieved by IVF; it is known that ICSI improves fertilization rate compared with IVF.[8]

However, when ICSI first introduced to authors in 1990, various concerns arose regarding the safety of this new technique, that is why this technique was done after getting the written informed consent from couples and prenatal diagnosis was initiated in children to be born after ICSI treatment.[9]

Preimplantation genetic diagnosis (PGD) is a technique used to determine the genetic defects in embryos created through IVF before their transfer to the uterus. Maternal age >35 years, patients with previous IVF treatment that resulted in trisomic conception, recurrent pregnancy loss, failed IVF treatments despite morphologically and high-quality embryo transfer, HLA-matched embryo selection for siblings, sex selection for specific diseases and cultural purposes are the main indication for PGD. Analyzing one or two of the blastomeres from day 3 embryos, cytotrophoblasts from the blastocyst-stage embryos, and polar bodies from the oocytes with five to 10 FISH probes provides useful information for PGD.

Patrizio has drawn attention to the risks about the situation that led to the ICSI (ICSI independent) and ICSI itself (ICSI dependent).[10] First ICSI dependent risk is biochemical and/or physical disorder of meiotic axis or ooplasm.[11-13] Second risk is injection area damage caused by variability of metaphase 2.[14,15] Third risk is injection of the sperm-associated foreign DNA and/or biochemical contamination.[16,17]

ICSI-independent risks not related to ICSI procedure are microinjection of sperm, which is bearing chromosomal abnormalities such as structural defects or aneuploidy, transferring of male factor related genetic defect like Yq deletion or cystic fibrosis mutations, structural defect of male gamete, sperm activating factor anomalies, mitochondrial DNA, and oocyte aging-related situations.[18]

In our study, 57 embryos from nine IVF patients, 183 embryos from 27 ICSI patients evaluated and there were no differences between IVF and ICSI groups in terms of aneuploidy rate. Among the 57 embryos from the IVF cases, the number of absolute abnormal chromosomes were as follows: X and Y chromosomes: 4 (12.9%), chromosome 13: 9 (29%), chromosome 16: 7 (22.5%), chromosome 18: 6 (19.3%), chromosome 21: 8 (25.8%), chromosome 22: 10 (32.2%). For the ICSI embryos: X&Y chromosomes: 18 (14%), chromosome 13: 34 (26.5%), chromosome 16: 23 (18%), chromosome 18: 23 (18%), chromosome 21: 26 (20.3%), chromosome 22: 31 (24.2%). The odds ratios for the difference between

IVF and ICSI for each chromosome were as follows: X&Y chromosomes: 1.53 (0.598-3.916), chromosome 13: 0.969 (0.443-2.122), chromosome 16: 0.709 (0.307-1.639), chromosome 18: 1.650 (0.650-4.188), chromosome 21: 0.777 (0.350-1.724), chromosome 22: 0.647 (0.311-1.348). Overall, no significant difference between two insemination procedures was seen 0.948 (0.678-1.324).

Odds ratios of the present study relationship between abnormal chromosomes and IVF-ICSI for chromosome XY, chromosome 13, chromosome 16, chromosome 18, chromosome 21, chromosome 22 are 1.53, 0.969, 0.709, 1.650, 0.777, 0.647, respectively. The total number of abnormal chromosomes between IVF and ICSI is 0.948.

There are studies in the literature showing that ICSI procedure does not cause an increased risk of chromosomal abnormalities compared with conventional IVF , as well as there are studies that claims the opposite.

One hundred and sixty-three couples referred for assisted reproductive technology and treated with subzonal insemination and ICSI in Bonduelle M *et al*'s study. They stated that major malformations incidence was not significantly different from general population after prenatal diagnosis and subsequent clinical follow-up of all children[9]: 24 after ICSI, 21 after SUZI , 10 after ICSI and SUZI; total 43 out of 55 children were tested prenatally. It is noteworthy that study is a prospective study, although low number of cases is a disadvantage of this study. However, preliminary data from this study showed no increase in the risk of anomalies for fetal karyotype after ICSI, so it is shown that there is no case to be concerned about ICSI. Similarly, prenatal testing for advanced maternal age showed no increased risk in terms of chromosomal abnormalities after ICSI.[19-21]

In contrast, few studies in the literature showed that there is an increased risk of chromosomal abnormalities after ICSI.[22,23] In't Veld *et al*. first reported that 33% of ICSI pregnancies had a chromosomal abnormalities identified by prenatal diagnosis, in which all five chromosomal abnormalities were on sex chromosomes. However, their study included a selection bias, as it was based on a referral for advanced maternal age for prenatal diagnosis. Additionally, the sample size was small and there was no information about parents' genetic status.[22] In another study, chromosomal aberrations (12.7%) were identified 9 of 71 fetuses after ICSI by prenatal cytogenetic analysis. Two out of nine cases were 47, XXY and three out of nine cases were autosomal trisomy.[23]

In our study, chromosome 22 was the most common chromosome containing aneuploidy in IVF group. It is followed by chromosome 13, 21 and chromosome 16 in descending order. The sex chromosomes (XY) were the least chromosomes containing aneuploidy. Chromosome 13 was the most common chromosome containing aneuploidy in ICSI group. It is followed by chromosome 22, 21, 16, and chromosome 18 in descending order. The sex chromosomes (XY) were the least chromosomes containing aneuploidy in ICSI group.

Comparative genomic hybridization is more effective than FISH for identifying chromosomally normal embryos, which may result in a higher clinical pregnancy rate and implantation rate after embryo transfer. Therefore, this situation is one of the restriction for the present study. The other one is relatively small number of the study.

ART pregnancies consist of conventional IVF and ICSI pregnancies. Studies cannot prove an idea that an increase risk of aneuploidy might be a result from limited (confined) placental mosaicism and persistent embryonic mosaicism when consider ART studies which found a high rate of aneuploidy in pregnancy. Studies have revealed that prevalence of CPM was not high in the ICSI group compared with conventional IVF group. In addition, CPM prevalence in ART pregnancy were similar in general population.[24,25] Although the majority of the studies showing that there is no increasing risk of chromosomal abnormalities in ICSI pregnancies, these concerns has been continuing because there is no prenatal and postnatal systematic chromosomal analysis for children arising from ICSI pregnancies and there are no studies about comparing the conventional IVF and ICSI with eliminating the risk factors contributing to abnormalities. In addition, high-risk situations such as study groups contain a small number of cases, ultrasonography detected chromosomal abnormalities, maternal age risk, and chromosomal abnormalities in mother or father, are known to be obstacles to clearly identify ICSI-associated risk of chromosomal abnormalities.

The relationship between male infertility and genetic abnormalities is known for a long time. But this relationship was more frequent in patients with azoospermia and severe oligozoospermia (less than 5 million sperm count/mL). Azospermia patients have significantly increased risk of aneuploidy compared with normospermic patients according to large number of sperm analysis report with the development of FISH analysis.[26,27]

As shown in most studies in the literature there is an opposite relationship between sperm quality and sperm aneuploidy. So, the low quality of sperm increase the chance of sperm abnormalities.[28-31] It has been reported that increased risk of aneuploidy in gamete seen in

men who are somatically normal but have an abnormal karyotype.[32] Intratesticular environment is thought to contribute aneuploidic gamete formation by disrupting chromosome segregation.[33] Male factor effect on aneuploidy risk are not known in this study, because patients infertility causes did not separate into male and female factors in IVF and ICSI patients. This situation can be interpreted as a limitation of this study. According to results of a prospective study, that compared children of couples who naturally get pregnant with the children of couples who get pregnant after ICSI procedure, showed that was a small but significant increased risk in terms of aneuploidy in ICSI group. An increased risk appeared to be equal in the autosomal and sex chromosomes.[34]

A study, which examined chromosomal anomalies in spontaneous abortion materials after ART, showed that there was no increased risk of ART-related chromosomal abnormalities. The only exceptions are patients who underwent ICSI due to male infertility. There was a significant increased risk of chromosomal abnormality in this study when patients who underwent ICSI due to male infertility group compared with the group has no male factor. Nevertheless, the authors stated that this increase risk is not related to ICSI procedure but related to the underlying parenteral risk. There were ICSI, conventional IVF and the control group in this study. The most common chromosomal abnormalities were trisomy and sex chromosome aneuploidy (second most common).[35] De-novo aberrations were found 1.6% or the tested ICSI children, which was significantly higher than in the general newborn population that 0.45% de-novo anomalies were reported previously. This higher rates was mostly due to a higher rate of sex chromosomal aberrations.[36] 1.6% de-novo anomalies (sex chromosomal and autosomal) in ICSI pregnancies with a mean maternal age of 33.5 years are significantly higher than the 0.87% de-novo anomalies expected for a mean maternal age of 35 years (no data under the age of 35 years were collected)[37,38] or than the 0.45% de-novo anomalies calculated on the basis of previous reports[39,40] at a maternal age of 33.5 years when compared with literature about data on prenatal diagnosis in the general population.

In contrast to many studies that revealed the relationship between male infertility and sperm chromosomal abnormalities, Wennerholm *et al.* stated that obstetric outcomes of ICSI pregnancies had not been affected by origin and quality of the sperm. They have published that obstetric outcomes of ICSI pregnancies were same with conventional IVF outcomes.[41] Therefore, it is clear that multicenter prospective double-blind randomized studies are needed in order to evaluate the risk of chromosomal abnormalities in IVF and ICSI pregnancies, the contribution of male infertility and parenteral risks.

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Conflicts of interest

There are no conflicts of interest.

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