Delayed intracytoplasmic sperm injection (ICSI) with trophectoderm biopsy and preimplantation genetic screening (PGS) show increased aneuploidy rates but can lead to live births with single thawed euploid embryo transfer (STEET)

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Abstract

Purpose The aim of this study was to report the results of IVF with trophectoderm biopsy and preimplantation genetic screening (PGS) following delayed intracytoplasmic sperm injection (ICSI).

Methods Patients undergoing IVF with PGS and delayed ICSI were included in the study. Indications for delayed ICSI included absent or poor fertilization via standard insemination or more than 50 % immature oocytes, noted post-cumulus stripping for standard ICSI procedure. Delayed ICSI was performed the day after retrieval due to absent or poor fertilization. The immature oocytes were kept in extended culture, and if demonstrated maturity, ICSI was performed. Primary outcome included fertilization rate and blastocyst stage formation, defined by the number of blastocysts for biopsy. Secondary outcome included aneuploidy rate and pregnancy outcomes following single thawed euploid embryo transfers (STEET).

Results Sixteen patients with delayed ICSI were included in the study. Twelve were due to poor fertilization and four secondary to immature oocytes. A total of 219 oocytes were retrieved; ten were frozen upon patient request, 168 had standard insemination, and 13 had routine ICSI on the day of retrieval. A total of 129 oocytes underwent delayed ICSI. Sixty-three (49 %) fertilized, 19 (14.7 %) reached blastocysts for biopsy; five of which were chromosomally normal (26.3 %). Three patients underwent

STEET of a delayed ICSI embryo; all three resulted in live births, including one embryo biopsied on day 8 of development.

Conclusion Fertilization failure or an excessive proportion of immature oocytes in an IVF cycle, necessitating delayed ICSI, showed equivalent fertilization and blast formation rates. With the implementation of trophectoderm biopsy and PGS, these embryos can lead to healthy live born babies.

Keywords Fertilization in vitro · Sperm injection · Intracytoplasmic · Fertilization · Delayed

Introduction

Intracytoplasmic sperm injection (ICSI) is a remarkable technique that has improved the outcomes of in vitro fertilization (IVF) since its successful debut in 1992 [1]. It was initially developed to assist fertilization in the case of severe male factor infertility or when normal appearing sperm failed to fertilize oocytes during IVF using standard insemination. Typically, patients with low sperm concentration, poor motility, or abnormal morphology are candidates for ICSI, as well as those undergoing preimplantation genetic diagnosis (PGD) for single gene disorders. While these remain the most common indications for ICSI, some centers employ the technique widely, even in cases of non-male factor infertility.

Fertilization failure during standard insemination in IVF can be a devastating clinical scenario. Re-insemination post fertilization failure, also known as “rescue ICSI” or “delayed ICSI,” is utilized in the event that minimal or no fertilization is noted post standard insemination [2]. Poor fertilization following insemination is most often secondary to failed sperm binding and penetration into the oocyte [3], allowing for another attempt at fertilization via ICSI. Previous studies have indicated that fertilization, blast formation, and pregnancy rates are

Capsule Fertilization failure or an excessive proportion of immature oocytes in an IVF cycle, necessitating delayed ICSI, showed equivalent fertilization and blast formation rates.

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considerably lower when ICSI is performed the day following retrieval compared to standard (day 0) ICSI and insemination [4]; however, live births have been reported indicating its potential usefulness [5]. In addition to cases of failed fertilization, delayed ICSI can be utilized when immature oocytes compromise a large percentage (>50 %) of the total egg yield and extended culture successfully develops eggs to the pre-fertilization stage [6]. Prior studies have shown an associated increased aneuploid rate with delayed ICSI [7, 8], with both cleavage stage and blastocyst biopsy. The objective of this study is to evaluate the outcome of cycles utilizing delayed ICSI in IVF cycles with trophoctoderm biopsy for preimplantation genetic screening (PGS).

Materials and methods

A global retrospective Institutional Review Board (IRB) approval was obtained (IRB S13-00389). All patients who required delayed ICSI and then underwent IVF with trophoctoderm biopsy and PGS at the New York University Fertility Center were included. Indications for delayed ICSI were absent or poor fertilization using standard insemination on day of oocyte retrieval (day 0) or a high rate of immature oocytes as determined by cumulus stripping in preparation for standard ICSI.

Patients underwent controlled ovarian stimulation via various protocols, including estrogen prime, micro dose lupron, or GnRH antagonist. Baseline ultrasounds were performed on day two of menstrual cycles. Patients with estrogen values less than 75 pg/ml and FSH less than 13.5 mIU/ml were initiated on gonadotropins until two lead follicles reached at least 18 mm in diameter. Oocyte maturation was induced using 256 or 512 µg of subcutaneous recombinant human chorionic gonadotropin (hCG), 5000 or 10,000 units of intramuscular urinary derived hCG or subcutaneous GnRH agonist (Lupron 2 mg) combined with one of the above hCG preparations (1000 units or 6 µg, respectively). Oocyte retrieval was performed 35 h post trigger injection.

Insemination or ICSI occurred 3 to 6 hours post retrieval. Insemination was performed by exposing oocytes to 15,000 to 40,000 sperm per milliliter in 75 µl of Global IVF media under oil. For patients undergoing standard ICSI, oocytes are denuded of cumulus cells using HTF Hepes buffered medium (Irvine Scientific) with hyaluronidase (Cutter Biological). The maturity of the oocyte was evaluated by confirming the absence of germinal vesicles and the presence of a polar body, indicating a metaphase II (MII) oocyte suitable for ICSI. If a high percentage of oocytes was found to be immature (>50 %), in meiosis I (M1), these immature oocytes were kept for extended culture (Global IVF media, LifeGlobal LLC) in IVF media for approximately 20 hours. Immature oocytes at the germinal vesicle stage were discarded and not

kept for extended culture. At the time of fertilization evaluation, if they appeared to be mature, delayed ICSI was performed. Fertilization evaluation for standard insemination and ICSI are routinely performed approximately 15 and 18 hours later, respectively. In cases of absent or poor fertilization (defined as greater than 75 % not fertilized) following standard insemination, “rescue ICSI” was performed at that time. Fertilization was evaluated in these oocytes 4 h post delayed ICSI (to confirm initial non-fertilization) as well as 18 hours later.

All fertilized oocytes were cultured in Global IVF Media (LifeGlobal LLC), and any embryos with progressive cellular division on the third day of culture underwent laser zona breaching (LZB). Embryos were re-evaluated on day five, six, seven, or eight (post insemination), and for those blastocysts deemed of adequate quality based on morphological assessment of grade using the trophoctoderm, biopsy for PGS was performed on that day. Blastocysts were vitrified in individual straws on the same day of biopsy, and removed cells were immediately vitrified and sent to Reprogenetics Inc (Livingston, NJ) for chromosomal analysis via comparative genomic hybridization (aCGH) or next generation sequencing (NGS). Until 2015, aCGH was the method used for PGS of trophoctoderm biopsy; however, due to technological advances, cycles occurring from 2015 and later had NGS performed. All other IVF procedures, including blastocyst culture and biopsy, remained the same.

Embryo transfers were performed in a subsequent frozen embryo transfer cycle using a programmed or natural cycle, with either intramuscular or vaginal progesterone (one patient) for luteal support. Single thawed euploid embryo transfers (STEET) were performed under ultrasound guidance, and followed by serum hCG test 9 days post transfer with subsequent blood and ultrasounds to follow.

Primary outcomes include fertilization and blastocyst formation rate. Fertilization was defined as presence of two pronuclei 20 hours post insemination or ICSI or evidence of cellular division 2 days after fertilization. Secondary outcome included aneuploidy rates as determined by PGS using array comparative genomic hybridization and pregnancy outcome. Data was collected retrospectively and statistical analysis using relative risk calculations performed using www.openepi.com.

Results

From 2003 to 2015, a total of 161 patients underwent an IVF cycle that resulted in the need for delayed ICSI. Of these, 16 patients had trophoctoderm biopsy with PGS and were included in the study. Indications for IVF with PGS were primary infertility ($n=11$), secondary infertility ($n=3$), male factor ($n=1$), and fertility preservation ($n=1$). The mean patient age was 38.3 ± 3.48 years (range 35–41), mean age at retrieval

(donors included) was 36.69 ± 4.33 years (range 27–41), with an average of 13.69 ± 7.33 eggs (range 6–34) was retrieved.

A total of 218 eggs were retrieved from 16 patients. One patient elected to freeze ten of her oocytes and undergo standard insemination with the remaining. A total of 168 oocytes from 12 patients initially had standard insemination, while 13 oocytes from four patients had ICSI performed on the day of retrieval see (Table 1). For those who had ICSI for the initial insemination, a combined total of 40 oocytes were retrieved. However, only 13 were mature oocytes (MII) suitable for ICSI; the remaining was kept for extended culture. The indication for these four patients to have routine ICSI performed included prior fertilization failure with standard IVF insemination ($n=2$), male factor due to a history of abnormal morphology ($n=1$), and patient preference ($n=1$). Both fresh ($n=13$) and frozen ($n=3$) sperm samples were used, donor sperm being the indication for frozen specimens.

From all eggs, standard insemination, routine ICSI, and delayed ICSI, a total of 32 blastocysts were biopsied, with all patients having at least one blastocyst for biopsy. Of these, 19 blastocysts that were biopsied were from embryos derived from delayed ICSI (25.40 % of fertilized delayed ICSI eggs). A total of nine out of 32 blastocysts biopsied were found to be euploid (28.1 %), and 23 were aneuploid (71.8 %). Seven patients (43.8 %) had euploid embryos from this cycle; two patients had two euploid embryos each, while the remaining had one euploid each. Ten patients did not have a transfer performed due to the absence of euploid embryos ($n=9$) or due to embryo banking for fertility preservation ($n=1$). Fourteen patients underwent autologous cycles while two were anonymous donor oocyte cycles.

Indications for delayed ICSI included fertilization failure after standard insemination with IVF ($n=14$) and low percentage of mature oocytes at time of retrieval ($n=4$). In the cases of extended oocyte culture for immaturity, the percent of mature oocytes ranged from 16 to 36 %; amongst four patients, a cumulative total of 20 M1 oocytes were left in culture, and 18 progressed to M2 oocytes suitable for delayed ICSI (see Table 2) The overall delayed ICSI blastocyst formation rate was 14.7 % (19 blastocysts per 129 oocytes), with a euploid rate of 26.32 % ($n=5$). Three patients underwent STEET of embryos from this cycle using (routine insemination or ICSI) that resulted in three pregnancies; one resulted in a first

Table 2 Outcomes shown by indication for delayed ICSI

	Failed fertilization	Extended oocyte culture
Patients	12	4
Total oocytes for delayed ICSI	111	18
Fertilization (2PN) (%)	56 (50.4 %)	14 (77.7 %)
Blastocyst for biopsy	15	4
Total euploid (%)	4 (26.7 %)	1 (25.0 %)

trimester miscarriage, one monochorionic-diamniotic ongoing twin gestation, and a third resulting in a second trimester miscarriage. Three different patients underwent STEET of delayed ICSI derived embryos, from day 6 ($n=1$), day 7 ($n=1$), and day 8 ($n=1$) biopsied blastocysts. All three transfers led to uncomplicated, full term, live births. All three transfers from delayed ICSI were secondary to poor fertilization.

Discussion

This descriptive study shows successful outcomes of delayed ICSI when used in cases of poor fertilization or in the presence of a high proportion of immature oocytes. Resulting euploid embryos demonstrated an excellent implantation potential.

One of our primary outcomes was fertilization; our rate of 54.26 % ($n=70/129$) is consistent with prior reports of delayed ICSI [9, 10] with 50.5 % ($n=56/111$) from poor fertilization (RR 0.46 CI [0.339–0.626] $p<0.001$) and 77.7 % ($n=14/18$) following extended oocyte culture for oocyte maturation (RR 1.41 CI [1.01–1.98] $p=0.063$). These rates are lower than those of normally timed attempts at fertilization, and determining the cause may be found in literature supporting a link between the need for delayed fertilization and oocyte DNA abnormalities. In cases of initial oocyte immaturity, chromosomal non-disjunction has been found [11] in eggs requiring greater than 4 h additional maturation time, with 80.6 % of the resulting day 3 embryos showed aneuploidy or mosaicism via FISH analysis (11). When the maturation and ICSI were delayed 24 h, the abnormality rate increased to 100 %. Likewise, it has been established that there are higher rates of aneuploidy and mosaicism in mature eggs

Table 1 Outcomes shown by method of initial insemination and delayed ICSI in same IVF cycle

	Standard insemination	Routine ICSI	Delayed ICSI
Patients	12	4	16
Total oocytes	168	13	129
Average oocytes	10.5 ± 7.86	3.25 ± 2.63	8.06 ± 3.87
Fertilization (2PN) (%)	42 (25.0 %)	10 (76.9 %)	63 (48.8 %)
Blastocyst for biopsy	8	5	19
Total euploid (%)	2 (25.0 %)	2 (40.0 %)	5 (26.3 %)

demonstrating delayed fertilization (8, 12). Potential causes of this improper chromosomal segregation range from alteration in oocyte activation, premature chromatin condensation, cytoskeleton changes, and organelle redistribution [12].

One of our secondary outcomes was the rate of blastocyst aneuploidy. We showed elevated aneuploidy rates with delayed ICSI, and these results parallel those of Emery et al., who found an aneuploidy rate of 61 versus 43 % for controls in embryos resulting from delayed ICSI for failed fertilization of immature oocytes, again using day 3 embryos and FISH [13]. Our work extends information on this topic by karyotyping embryos cultured to day 5 and the use of more sophisticated DNA analytics in the form of aCGH and NGS.

We have also shown that aneuploidy rates are similar with delayed ICSI as compared to embryos resulting from normally timed fertilization in the subset of patients needing delayed ICSI. These rates comparable to prior studies, but higher than reported in similarly aged women undergoing IVF-PGS with normally timed fertilization [13, 14]. The potential genetic causes of fertilization delay discussed above are the same factors that can go on to alter proper cell division leading to the development of an aneuploidic embryo [13]. The basis for abnormal chromosomal function could be due to faulty cumulus cell and oocyte interactions, a dysregulated cyclic AMP pathway, and abnormal calcium modulation [15].

To this point, we have discussed the abnormal oocyte and its role in delayed fertilization, but essential errors in spermatocyte chromosome segregation should be considered. Oocyte non-disjunction related to advancing maternal age has clearly been shown to contribute to aneuploidy, as well as with extended culture of meiosis II oocytes [16]. However, evidence for the association of aneuploidy and male infertility is growing as well. Sub-fertile men have higher rates of aneuploid offspring secondary to gametes with higher rates of non-disjunction [17]. Data showing increased chromosomal anomalies in sperm with impaired zona binding capabilities may imply that the zona pellucida acts selectively to prevent sperm with chromosomal abnormalities in some cases [18].

Some suggest that although fertilization and embryo development occurs with delayed ICSI, implantation and pregnancy rates are reduced [19], indicating poorer quality of embryos. Dal Canto et al. studied 1096 cases where the stimulation protocol was geared towards retrieving immature eggs for in vitro maturation; a total of 6113 oocytes were obtained [20]. After maturation, implantation rates were lower than in embryos resulting from eggs mature on retrieval day. Certainly, potential for implantation is multifaceted; however, we have found that by controlling for aneuploidy using trophectoderm biopsy and PGS, an excellent rate of implantation can be expected. The successful outcomes in our study with three euploid embryos following delayed ICSI prove that the likely negative impact of extended oocyte culture and failed fertilization can be bypassed with PGS.

Given its infrequent use, the number of our patients who had delayed ICSI with subsequent PGS performed was low, leading to a small sample size. However, three pregnancies were seen from blastocysts biopsied and cryopreserved on day six, seven, and eight. No meaningful statistical analysis could be done with such a small cohort, so all conclusions must be viewed cautiously. For patients with poor fertilization or a low proportion of mature oocytes, delayed ICSI with trophectoderm biopsy and PGS may be a viable option to help improve the chances of having a live birth. In addition, given that this is a retrospective study, many factors such as variations in stimulation protocol and trigger medications may contribute to oocyte maturity, in addition to other unknown factors that may affect overall outcomes. The standard in our center is to culture embryos until the seventh day post fertilization; however, in this case, given that delayed ICSI occurred on the day after initial fertilization, embryos were left to culture until the eighth day. Only one patient had one embryo that was of biopsy quality. This study is one of the first to describe a pregnancy from trophectoderm biopsy from delayed ICSI blastocyst cultured to the eighth day post retrieval; indicating that extended culture in this case had no apparent adverse impact on pregnancy potential.

Conclusion

Although routine use of delayed ICSI followed by blastocyst biopsy and PGS is not always successful, in certain circumstances it may be useful and can lead to healthy live births. Within the same cycle, our patients demonstrated similar aneuploidy rates whether fertilization occurred on the day of or day after retrieval. Although lower fertilization rates are present, delayed ICSI may give some patients the opportunity for a successful IVF cycle where they otherwise would not.

Compliance with ethical standards A global retrospective Institutional Review Board (IRB) approval was obtained (IRB S13-00389).

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