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ASSISTED REPRODUCTION TECHNOLOGIES

Should ICSI be recommended routinely in patients with four or fewer oocytes retrieved?

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Abstract
Purpose To determine if patients with a low response to controlled ovarian hyperstimulation during IVF benefit from intracytoplasmic sperm injection (ICSI)
Methods Retrospective analysis of 350 IVF cycles in which four or fewer oocytes were retrieved. Severe male factor cases were excluded from analysis. Conventional insemination (CI) and ICSI were compared, with primary outcome measures of fertilization rate, implantation rate, clinical pregnancy rate per embryo transfer, and pregnancy loss rate.
Result(s) Fertilization rates per oocyte retrieved for CI and ICSI were comparable (51.5% vs. 51.8%). Parallel implantation rates (22% vs. 25%), clinical pregnancy rates (32.8% vs. 33.3%), and loss rates (26.7% vs. 39.5%) were also noted. No difference in cancelled cycles was reported.
Conclusion(s) Our results demonstrate that in the presence of normal semen parameters, low egg number is not an indication to perform ICSI.

Capsule In the presence of normal semen parameters, low response to ovarian hyperstimulation is not an indication to perform ICSI over conventional insemination.

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Introduction

Male factor infertility is a well-established indication for intracytoplasmic sperm injection (ICSI) [1–3]. Assisting fertilization allows bypass of the zona pellucida with a single spermatozoon [4]. Since the report of the first human pregnancies achieved by this technique, ICSI has been applied extensively worldwide. There is still conflict, however, regarding the use of ICSI in couples with non-male factor infertility or unexplained infertility.

ICSI has been associated with high fertilization and pregnancy rates regardless of sperm concentration, motility or morphology [5–7]. The rate of ICSI procedures in cycles without male factor infertility has increased progressively during the last 7 years, comprising 39% of all ICSI cases in 2000 and rising to greater than 50% of cases in 2006. It seems as though ICSI has been universally promoted and is increasingly replacing conventional insemination due to its many benefits and lack of definable drawbacks [8].

Indications for performing ICSI include male factor infertility, history of failed or low fertilization rate, unexplained infertility, couples who lack a history of pregnancies, and immunological infertility [6, 7, 9–11]. A subset of patients in whom the risk-benefit of performing ICSI is unclear is low responding patients. An association between lower number of retrieved oocytes and lower fertilization rates and IVF outcome has been described [12, 13]. Additionally, fertilization failure is a common finding when less than 3 oocytes are retrieved [14] The high fertilization rates, small numbers of fertilization failures, and lack of apparent long term drawbacks make ICSI an appealing solution for IVF [15, 16]. Hence, it has

been proposed that ICSI could improve fertilization rates and the likelihood of successful embryo transfer when the number of recovered oocytes is low [17]. We decided to engage in the comparison of outcome of two fertilization techniques in patients who demonstrate a low response to gonadotropin stimulation, without any indication for ICSI.

Materials & methods

Patient data

A total of 350 IVF cycles with a low response to stimulation at Reproductive Medicine Associates of New York were retrospectively analyzed. The database was searched for IVF cycles performed from September 2002 and March 2009 based on number of retrieved oocytes and insemination technique. Pertinent charts were reviewed for cycle data and outcomes. All cycles in which four or fewer oocytes were retrieved following controlled ovarian hyperstimulation were included. Cases with severe male factor infertility were excluded from this analysis. ICSI was performed based on physician preference due solely to low oocyte yield. Institutional Review Board approval was obtained for the performance of this retrospective analysis.

Controlled ovarian hyperstimulation and oocyte retrieval

Controlled ovarian stimulation was achieved using GnRH antagonist, Down-Regulation, Microflare and Estrogen Priming protocols. Patients were serially monitored for estradiol and progesterone levels, as well as count and size of follicles using transvaginal ultrasound in the office. Patients were administered human chorionic gonadotropin (hCG) injection with the presence of two >17 mm follicles. Oocyte retrieval was performed under conscious sedation using a transvaginal ultrasound probe and needle aspiration 36 h after administration of hCG.

Gamete preparation, fertilization and embryo culture

Fresh ejaculated semen samples were provided on the day of vaginal oocyte retrieval (VOR). These samples were evaluated for sperm count; >20 million motile sperm were considered adequate for CI. Semen samples were washed and processed for CI or ICSI. Conventional insemination was performed 4–6 h after VOR. One to three oocytes with surrounding cumulus complex were placed in 50 μL drops of media with approximately 50,000 motile sperm. For cases undergoing ICSI, oocytes were denuded of the cumulus complex 3–4 h after VOR using hyaluronidase. Oocytes were assessed for maturity by the presence of an expelled first polar body. Sperm were selected based on morphology and motility, the mitochondrial tail was disrupted with the injection pipette, and the single sperm was injected into the 3 o'clock position of the mature oocyte 5–6 h after VOR. All metaphase I oocytes were discarded.

Assessment of embryo quality for transfer

Fertilization was confirmed 16–18 h following CI or ICSI by the presence of two pronuclei in the zygote. Oocytes that failed to fertilize after conventional insemination were assessed for maturity. Conventionally inseminated oocytes that demonstrated one pronucleus were maintained in culture and evaluated the following day. Embryos were assessed daily following fertilization until Day 3. Assisted hatching using Tyrode's acid was performed on embryos transferred on Day 3. Blastocyst transfers were not performed due to the low number of oocytes retrieved per cycle. The number of embryos to be transferred was based upon embryo availability and subjective morphologic appearance of the embryos (including blastomere size, fragmentation, multinucleation). Cycle cancellation was determined upon fertilization failure or arrest of embryo development after VOR.

Determination of clinical pregnancy

Pregnancy test was performed on day 11 following embryo transfer through assessment of quantitative beta-hCG. Clinical pregnancy was defined as the presence of an intrauterine gestational sac on transvaginal ultrasound 21 days after embryo transfer. Pregnancy loss was defined as declining quantitative beta-hCG after the diagnosis of clinical pregnancy was made.

Statistical analysis

IVF cycles were analyzed based on insemination technique (conventional or ICSI). Data was analyzed using Analyze It® Microsoft Excel software. Statistical analysis of continuous variables was performed using Kruskall Wallis and one-way analysis of variance (ANOVA). Categorical data was analyzed using the chi-square test. Statistical significance was defined as $P< 0.05$.

Results

Conventional insemination was used for fertilization in 179 cycles and ICSI was performed in 171 cases. Patient demographics were similar between both groups, with a mean age of 39.1 and 39.6, respectively. Additionally, baseline FSH levels were not different (12.1vs 12.4 IU/L). Peak estradiol levels and the number of retrieved oocytes were not different between the two insemination methods. Fertilization rates per oocyte retrieved for CI and ICSI were comparable (51.5% vs. 51.8%). The number of embryos available for transfer per patient was 1.8 and 1.7 respectively. Parallel implantation rates (22% vs. 25%), clinical pregnancy rates (32.8% vs. 33.3%), and loss rates (26.7% vs. 39.5%) were also noted. The number of cancelled cycles prior to embryo transfer was not different (Table 1).

In our series, failure to fertilize occurred in 16.2% (29/179) and 10.5% (18/171) of CI and ICSI cases respectively (p=0.161). The immaturity rate of day 0 oocytes allocated to ICSI was 23.4% (133/169). The immaturity rate of oocytes allocated to CI was unknown until day 1, which was noted to be 7.2% (42/587). Fertilization rates of only mature oocytes allocated to CI or ICSI were 55.4% (302/545) and 67.7% (295/436 (p=0.001). The fact that conventionally inseminated oocytes are not assessed until day 1 of culture, may explain the relatively low immaturity rate, allowing immature aspirated oocytes to mature overnight.

We further analyzed whether a difference in outcome would be noted based on specific number of oocytes aspirated. Tables 2 and 3 describe the cases by number of oocytes retrieved and outcome related to clinical pregnancy and cancellation rates, respectively. No differences were noted for these variables regardless of number of aspirated oocytes (Table 3).

Discussion

Treatment of the "poor responder" has become an enormous challenge in our field. A variety of protocols have been applied for COH to try to enhance response for women known to be poor responders. Micromanipulation techniques, such as assisted hatching, have also been clinically applied in this subset of patients in hope of enhancing implantation rates. Previous studies have demonstrated higher fertilization rate and improved outcomes after ICSI procedures [18]. The objective of this study was to determine if patients with a poor response to gonadotropin stimulation benefit from undergoing ICSI.

Poor response to COH with low oocyte retrieval has an estimated prevalence of 9–24% of IVF patients. Though definitions of poor response vary with regard to number of oocytes retrieved, many studies have suggested that low responders have lower fertilization rates, pregnancy rates, and implantation rates after an IVF cycle [13, 19]. Complete fertilization failure, which occurs in approximately 16% of IVF cycles, was significantly more frequent in cycles that resulted in 3 or fewer oocytes [14, 20]. Though it has been suggested that 6–10 oocytes is the ideal quantity to optimize the number of embryos ultimately transferred [21], retrospective analyses of poor response cycles and subsequent fertilization and pregnancy rates suggest that embryo transfer is still possible in this patient population and cycles should not be cancelled [19, 22, 23]. While there have been prospective trials to compare IVF outcomes in CI versus ICSI, women with poor ovarian response [24] or previous low responders [25] have had cycles cancelled or have been excluded. In couples without poor response to COH, ICSI has no proven benefit over CI and is a more invasive and expensive technique [24–28]. Prospective randomized trials studying low response to COH have not been performed.

Table 1 Demographics and clinical outcome of CI and ICSI cases

	CI[a] (n=179)	ICSI[b] (n=171)	p value[f]
Age (yrs)	39.1±4.1	39.6±3.6	0.182
FSH[c] (IU/L)	12.1±6.8	12.4±6.8	0.645
Peak E2[d] (pg/mL)	1092.3±512	1017.8±432	0.144
Oocytes retrieved (n)	3.3±0.9	3.3±0.8	0.598
Embryos transferred (n)	1.8±0.8	1.7±0.8	0.339
Fertilization rate	51.5% (302/587)	51.8% (295/569)	0.915
Implantation rate	22% (54/246)	25% (54/219)	0.502
Cancellation rate	23.5% (42/179)	24.6% (42/171)	0.908
Clinical pregnancy rate/ET[e]	32.8% (45/137)	33.3% (43/129)	0.963
Loss rate	26.7% (12/45)	39.5% (17/43)	0.291

[a] *CI* conventional insemination

[b] *ICSI* intracytoplasmic sperm injection

[c] *FSH* follicle stimulating hormone

[d] *E2* estradiol

[e] *ET* embryo transfer

[f] Kruskall-Wallis and one-way ANOVA used for continuous variables; chi-square test for categorical variables

Table 2 Number of CI and ICSI cases based on oocytes retrieved

Cases	Conventional insemination	ICSI[a]
1 oocyte	7	4
2 oocytes	29	28
3 oocytes	50	47
4 oocytes	93	92
TOTAL	179	171

[a] *ICSI* intracytoplasmic sperm injection

Staessen et al. used sibling oocytes for comparison of CI and ICSI and did not demonstrate a significant difference between these techniques in terms of cleavage or embryo quality, though their study was limited by sample size [18]. In fact, Taylor et al. demonstrated a significantly higher fertilization rate with CI when compared to ICSI (53.2% vs. 41.1%, $p<0.0001$) in sibling oocytes [7]. On the other hand, a variety of studies comparing ICSI with CI suggest higher fertilization rates and pregnancy outcomes with this more complex assisted reproductive technology [4, 15, 16, 18, 29]. Recently, Ou et al. published a case–control study that showed significantly higher fertilization rates for ICSI in low response cycles and a favorable trend for ICSI in clinical pregnancy rate, implantation rate and live birth rate [23]. This study, however, was limited by small sample size. Many other retrospective analyses of low response to COH have shown no benefit to using ICSI over CI in low responder patients with fertile sperm [20, 22, 30]. Our study demonstrates overall parallel clinical outcome, with similar fertilization rates, number of embryos available for transfer, clinical pregnancy and cancellation rates between both groups.

Decreased incidence of total fertilization failure has been described after ICSI. This result is due to ensured sperm entry into the oocyte, thereby bypassing the zona pellucida and acrosome reaction necessary for in vivo fertilization or CI [4, 16, 31]. On the other hand, others have theorized that using ICSI may bypass natural selection methods and choose abnormal sperm, resulting in poor outcomes. In our analysis, total fertilization failure was not lower in oocytes allocated to ICSI.

Maturation of the oocyte is proportional to the number that will ultimately fertilize [32]; at least 1 h is needed after polar body extrusion for appropriate oocyte maturation and development [33]. ICSI is limited by the oocyte maturity required for injection, whereas CI may allow immature oocytes to complete maturation in culture while awaiting fertilization. It would seem, therefore, that ICSI would be more limiting in patients with low response, given the probability that any oocytes retrieved would be within the metaphase II window of maturity for this procedure. In our series, the immaturity rate of day 0 oocytes allocated to ICSI was 23.4%. The immaturity rate of oocytes allocated to CI was unknown until day 1, which was noted to be 7.2%. When analyzing fertilization rates of only mature oocytes allocated to CI or ICSI we encountered a significant difference between both groups (55.4% vs. 67.7%) ($p=0.001$). This may be explained by the fact that a significantly higher number of immature oocytes are discarded and not allocated to undergo ICSI, after hyaluronidase exposure. We further analyzed whether a difference in clinical pregnancy rate and cancellation rates would be noted based on the specific number of retrieved oocytes. We did not encounter a significant difference in these outcomes regardless of the number of retrieved oocytes.

There is a lack of randomized controlled trials studying ICSI in poor response cycles. Clinical management has been predominantly shaped by anecdotal reports and retrospective analyses of series notable for low sample size. The objective of this analysis was to determine if patients with low response to gonadotropin stimulation benefit from the use of ICSI for insemination. Based on our results, we conclude that couples with normal semen parameters do not require that ICSI be performed for oocyte number alone. Any potential benefit of "assisting" fertilization may be outweighed by stripping oocytes of

Table 3 Clinical pregnancy per transfer and cancellation rate by oocytes retrieved

Number of oocytes	Clinical pregnancy/ET[a]			Cancellation		
	CI[b]	ICSI[c]	*p*-value[d]	CI[b]	ICSI[c]	*p*-value[d]
1–2 oocytes	30% (7/23)	37% (7/19)	0.8144	36% (13/36)	41% (13/32)	0.6933
3 oocytes	41% (15/37)	26% (10/37)	0.2525	26% (13/50)	21% (10/47)	0.8651
4 oocytes	29% (22/75)	36% (27/76)	0.5229	19% (18/93)	17% (16/92)	0.8769

[a] *ET* embryo transfer

[b] *CI* conventional insemination

[c] *ICSI* intracytoplasmic sperm injection

[d] Chi-square test performed for statistical analysis

their cumulus complex prematurely and preventing normal fertilization of late maturing oocytes.

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