Live birth rates using conventional in vitro fertilization compared to intracytoplasmic sperm injection in Bologna poor responders with a single oocyte retrieved

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Received: 6 February 2015 / Accepted: 4 March 2015 / Published online: 11 March 2015
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Abstract

Purpose To compare reproductive outcomes following conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in poor responders fulfilling the Bologna criteria, with a single oocyte retrieved.

Methods The present retrospective study included 243 Bologna poor responders with a single oocyte retrieved, who were categorized into three groups, depending on the fertilization method and semen quality (IVF non-male factor-IVF/NMF $n=101$; ICSI non-male factor ICSI/NMF $n=50$; ICSI male factor-ICSI/MF $n=92$).

Results In IVF/NMF, ICSI/NMF and ICSI/MF similar fertilization rates [65.3, 66, 58.7 %, respectively], proportions of embryo formation [63.4, 60, 53.3 %, respectively], proportions of good quality embryos [54.7, 56.7, 57.1 %, respectively], implantation rates [8.9, 10, 8.2 % respectively] and live birth rates per oocyte retrieval [5.0, 4.0, 3.3 %, respectively] were observed. Degeneration rate of oocytes due to mechanical damage was significantly higher after ICSI in the ICSI/

NMF and ICSI/MF groups (8 and 6.5 %, respectively) compared to IVF/NMF (0 %) ($p=0.02$).

Conclusions Conventional IVF and ICSI are associated with similar reproductive outcomes in poor responder patients with a single oocyte retrieved. Therefore, the choice of fertilization method should be based primarily on semen quality, in combination with the patient's previous history. A randomized controlled trial should be performed to confirm this study's findings that conventional IVF and ICSI have similar reproductive outcomes in poor responders.

Keywords IVF · ICSI · Single oocyte · Poor responders · Bologna criteria · Live birth

Introduction

The introduction of intracytoplasmic sperm injection (ICSI) revolutionized assisted reproduction technologies [1, 2], making feasible the treatment of nearly all types of severe male infertility.

Despite an ongoing debate about the optimal fertilization method for couples undergoing ART treatment [3, 4], there seems to be a progressive global increase in the use of ICSI [5]. ICSI represented 60.6 % of ART cycles performed worldwide in 2004 [6], further increasing to 66 % in 2006 [7]. The latest data from European registers show that in central and western Europe ICSI was used in 75 % of cases in 2010 [8]. These figures suggest a more liberal application of the technique [9], which seems to be associated with professional strategies and/or personal preferences rather than an increase in male infertility that would mandate ICSI application [10].

ICSI is of proven benefit in male factor infertility and in cases with previous low fertilization, total fertilization failure

Capsule Conventional IVF and ICSI are associated with similar single oocyte retrieved and normal semen parameters.

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or high percentage of dispermy after conventional IVF with normal semen. However, ICSI has also been considered for non-male factor infertility couples [11–13], those with borderline sperm parameters [14], unexplained infertility [15–17], tubal infertility [18, 19], polycystic ovary syndrome [20], poor responders [21, 22], endometriosis-associated infertility [23], or couples with a high proportion of immature oocytes [24]. Some researchers have gone as far as proposing ICSI for all in-vitro fertilization cases [3, 25]. Nevertheless, there is a lack of evidence that ICSI improves fertilization and embryo implantation in the presence of normal sperm compared to conventional IVF [26–28], suggesting that the use of ICSI in all patients might not be justified [4, 29].

It is not yet clear whether this is also true for women with a small number of retrieved oocytes [30–32] or those lying at the worst end of the poor response spectrum in whom only a single oocyte is retrieved [33].

The aim of the present retrospective study was to compare ICSI and conventional IVF in Bologna poor responder patients with a single oocyte retrieved in the presence of male or non-male factor infertility.

Materials and methods

Patient population and inclusion criteria

The present retrospective study included 243 women, who underwent assisted reproduction treatment from May 2009 until December 2012.

In order for patients to be included in the study they had to fulfill the Bologna criteria [34] for the definition of poor ovarian response. Patients should have at least two of the following: advanced female age, a diminished ovarian reserve as shown by low antral follicle count ($\text{AFC} \leq 5$) or increased basal FSH (>12 IU/L), and one or more failed previous IVF/ICSI cycles with poor ovarian response in which ≤ 3 COCs had been retrieved using a high gonadotrophin dose. Moreover, patients should have a single oocyte retrieved at oocyte pick-up in the current cycle.

These patients were retrospectively divided into three groups depending on the method of fertilization and on semen quality as follows: IVF non-male factor-IVF/NMF $n=101$; ICSI non-male factor-ICSI/NMF, $n=50$; ICSI male factor-ICSI/MF $n=92$.

According to WHO reference values [35], normal semen parameters were: sperm concentration $\geq 15 \times 10^6/\text{ml}$, progressive motility $\geq 32\%$. Morphology criteria of WHO 4th edition [36] with reference value $\geq 14\%$ normal forms were used for semen assessment prior to 2010. Strict morphology criteria described in WHO 5th edition [35] with reference value $\geq 4\%$ normal forms were applied from 2010 onwards.

Patients who opted to undergo natural cycle IVF or minimal ovarian stimulation due to personal preferences and did not

fulfill the Bologna criteria for poor ovarian response, as well as cases with frozen-thawed sperm, or testicular biopsy were excluded from the study. Patients could enter the study only once.

Ovarian stimulation and embryology procedures

Patients underwent ovarian stimulation using GnRH agonist long or short protocol, antagonist protocol [37] or modified natural cycle [38]. Final oocyte maturation was triggered using 10,000 IU hCG (Pregnyl; MSD, Oss, The Netherlands) when the mean follicular diameter was ≥ 16 –17 mm. Oocyte retrieval was performed by transvaginal ultrasound-guided double lumen needle aspiration 35–36 h after hCG administration. Due to the presence of only one follicle in most cases, repeated follicular flushing was performed when the oocyte was not retrieved after the initial puncture to maximize chances of recovery.

ICSI was used in all cases with suboptimal sperm quality. In cases with normal semen parameters, the decision about the method of fertilization was taken by the couple on the day of oocyte retrieval. The couples were informed about the presence of normal sperm and its suitability for conventional IVF, and were fully explained about the indications for IVF and ICSI, advantages and disadvantages of the two methods, about the increased cost of ICSI, about the invasive nature of ICSI and current concerns regarding the health of the offspring, as well as about the possibility of fertilization failure using either method, which in the case that a single oocyte is retrieved equals to no embryos available for embryo transfer.

Oocytes were fertilized with either conventional IVF using an insemination concentration of 150,000 /ml or ICSI. All ICSI procedures in the present study were performed by the same embryologist to reduce technique-related variability. Fertilization was assessed 16–18 h post insemination.

Embryos were assessed based on morphological criteria (number, size and shape of blastomeres, degree of fragmentation, multinucleation, appearance of cytoplasm), and were categorized in four grades [grade 1 (highest) to grade 4 (lowest)]. Embryos with 4 cells on day 2 and embryos with 6–8 cells on day 3, and $<20\%$ fragmentation were regarded as good quality embryos (grades 1 and 2).

Embryo transfer was performed 2 or 3 days after oocyte retrieval depending on the Unit's daily schedule.

Luteal phase support was performed by administering 600 mg micronized progesterone (Utrogestan, Laboratoires Besins International SA, France) from the day of embryo transfer until the 10th week of gestation, if pregnancy occurred.

Ultrasound and laboratory assays

All ultrasound measurements were performed using a 7.5 or 6 or 5 MHz vaginal probe (Sonoline Adara, Siemens). An

Immolute analyzer and commercially available kits (DPC, Los Angeles, CA) were used for FSH, LH, oestradiol and progesterone measurements. Analytical sensitivities of the assays were 0.1 mIU/ml for FSH, 0.1 mIU/ml for LH, 15 pg/ml for oestradiol and 0.2 ng/ml for progesterone. Intra- and inter-assay precision (expressed as coefficients of variation) were 2.6 and 5.8 % for FSH, 5.9 and 8.1 % for LH, 6.3 and 6.4 % for oestradiol and 7.9 and 10 % for progesterone.

Outcome measures

The primary outcome measure was achievement of live birth. Secondary outcomes included fertilization and embryo formation rates, embryo quality, number of oocytes with single pronucleus (1PN) or multiple pronuclei (3PN), oocyte damage rates, implantation rates (number of gestational sacs with positive heartbeat per embryos transferred, assessed at 7 weeks of gestation) and early pregnancy loss (percentage of positive hCG not reaching ongoing pregnancy).

Statistical analysis

Binary data and continuous data were analyzed for statistical significance ($p < 0.05$) using chi square test, and one-way ANOVA with Bonferroni post-hoc analysis, respectively.

A logistic regression was performed to assess the effects of stimulation protocol and day of transfer on the primary outcome.

Results

The study included 243 poor responder patients with a single oocyte retrieved, who were treated as follows: IVF/NMF $n = 101$, ICSI/NMF $n = 50$. ICSI/MF $n = 92$.

Similar baseline characteristics were observed between the above groups (Table 1), which were indicative of low ovarian reserve. Moreover, sperm concentration, motility and morphology in the IVF/NMF and ICSI/NMF groups were significantly better ($p < 0.0001$) compared to ICSI/MF (Table 1).

Proportion of patients with previous fertilization failure using conventional IVF was similar in the non-male factor groups [12.9 versus 6 % in IVF/NMF and ICSI/NMF, respectively]. This proportion was significantly higher ($p = 0.006$) in IVF/NMF compared to ICSI/MF (2.2 %) (Table 1).

Distribution of patients undergoing GnRH antagonist protocol, GnRH agonist short protocol, GnRH agonist long protocol or modified natural cycle (Table 1), as well as the proportion of patients with embryo transfer performed on Day 2 and Day 3 (Table 2) were similar between the groups compared.

In IVF/NMF, ICSI/NMF and ICSI/MF similar fertilization rates [65.3, 66, 58.7 %, respectively], proportions of embryo

formation [63.4, 60, 53.3 %, respectively], proportions of good quality embryos [54.7, 56.7, 57.1 %, respectively], proportions of oocytes with three pronuclei [7.9, 2, 2.2 %, respectively] and one pronucleus [2.9, 2, 0 % respectively] were observed. Degeneration rate of oocytes due to mechanical damage was significantly higher after ICSI in the ICSI/NMF and ICSI/MF (8 and 6.5 %, respectively) compared to IVF/NMF (0 %) ($p = 0.02$) (Table 2).

Live birth rates per oocyte retrieval [5.0, 4.0, 3.3 %, respectively] and implantation rates [8.9, 10, 8.2 % respectively], were similar between IVF/NMF, ICSI/NMF and ICSI/MF (Table 2).

The logistic regression model (pseudo $R^2 = 0.191$) was not statistically significant ($\chi^2 = 2.181$, $p = 0.82$), and showed that the type of protocol ($p = 0.924$) and day of transfer ($p = 0.426$) did not have a confounding effect on live birth.

Discussion

The present study provides evidence that ICSI and conventional IVF are associated with similar reproductive outcomes in poor responder patients in whom a single oocyte is retrieved at oocyte pick-up. Similar fertilization rates, embryo quality, and pregnancy rates were observed between IVF and ICSI in the presence of normal sperm, suggesting that the method of insemination does not appear to influence embryo developmental competence and the probability of pregnancy. Reproductive outcomes were also similar in patients with suboptimal semen quality undergoing ICSI.

To the best of our knowledge, there is only one previous study comparing IVF and ICSI in poor responder patients with a single oocyte retrieved at oocyte pick-up and non-male infertility [33]. In that study, fertilization rates and pregnancy rates were similar between IVF and ICSI, respectively. Interestingly, the authors report that no pregnancies were established when ICSI was used. In addition, no definition of poor response was provided, and the reported mean basal FSH levels of women included in that study were rather low (< 10 IU/l) [33]. In contrast, patients in our study were classified as poor responders according to the Bologna criteria, lying at the worst end of the poor response spectrum, as shown by the increased age, high basal FSH levels and low antral follicle count.

Regarding the potential introduction of bias, given the retrospective nature of the present study, we showed that the day of transfer and the type of protocol did not have a confounding effect on live birth, using logistic regression analysis. In addition, the proportion of patients with previous fertilization failure using conventional IVF was similar in the non-male factor groups (IVF/NMF and ICSI/NMF) which are of main interest, therefore minimizing the bias possibly associated with the choice of fertilization method in these patients.

Table 1 Demographic and baseline characteristics of poor responding women included in the study, ovarian stimulation protocols used, and sperm characteristics

	IVF/NMF N=101	ICSI/NMF N=50	ICSI/MF N=92	Overall p
Women's characteristics	mean±SD (95 % CI)			
Age (years)	40.9±3.7 (40.1–41.6)	41±4.7 (39.7–42.2)	41.1±4.1 (40.3–41.9)	NS
BMI (kg/m ²)	24.0±4.3 (22.9–25.2)	23.0±2.7 (22.2–23.7)	23.3±3.7 (22.5–24.2)	NS
Years of infertility	3.0±2.6 (2.4–3.8)	4.4±4.4 (3.2–6.0)	4.6±5.2 (3.4–5.9)	NS
Number of previous attempts	3.2±2.5 (2.7–3.8)	3.1±2.5 (2.5–3.9)	3.7±2.8 (3.1–4.3)	NS
Antral follicle count (AFC)	2.9±1.4 (2.6–3.2)	2.9±1.3 (2.5–3.3)	3.0±1.5 (2.6–3.4)	NS
Basal FSH (mIU/ml)	18.4±10.3 (15.6–21.6)	15.7±7.3 (13.4–18.1)	15.5±8.4 (13.6–17.5)	NS
Gonadotrophin dose (IU) in conventional COS protocols	3017.9±1060.0 (2535.4–3500.4)	2972.5±1440.0 (2175.1–3769.9)	2990.8±851.0 (2597.1–3244.5)	NS
Ovarian stimulation protocols	% (n) (95 % CI)			
GnRH antagonist	28.7 (29) (20.8–38.2)	30 (15) (19.0–43.8)	31.5 (29) (22.9–41.6)	NS
GnRH agonist short	3 (3) (0.7–8.7)	4 (2) (0.3–14.2)	2.2 (2) (0.1–8.1)	NS
GnRH agonist long	2 (2) (0.1–7.4)	2 (1) (0.0–11.5)	1.1 (1) (0.0–6.5)	NS
Modified natural cycle	66.3 (67) (56.6–74.8)	64 (32) (50.1–75.9)	65.2 (60) (55.03–74.18)	NS
Patients with fertilization failure using IVF in previous cycle	12.9 (13) (7.7–20.8)	6 (3) (2.1–16.2)	2.2 (2) (0.6–7.6)	0.018*
Sperm characteristics	mean±SD (95 % CI)			
Sperm Concentration (×10 ⁶ /ml)	87.6±57.7 (75.9–100.1)	59.1±45.7** (47.7–72.1)	6.6±5.9** (5.5–7.8)	<0.0001
Sperm Motility (%)	63.3±12.7 (60.7–66.1)	64.1±12.9 (60.5–67.7)	41.6±24.7** (36.7–47.2)	<0.0001
Sperm Morphology (% normal forms) prior to 2010	37.3±17.2 (33.6–41.1)	31.4±14.3 (26.7–36.2)	19.3±9.4** (17.1–21.6)	<0.0001**
Sperm Morphology (% normal forms) 2010 onwards	31.7±2.8 (29.2–34.3)	25±9.2** (19.4–30.6)	6.2±4.9** (4.15–8.27)	<0.0001**

Single asterisk denotes significant difference in the proportion of patients with previous fertilization failure using chi-square and Bonferroni post-hoc test (p level at 0.017 following Bonferroni correction). $P_{\text{chi-square}}=0.018$; $P_{\text{IVF/NMF vs ICSI/MF}}=0.006$, $P_{\text{IVF/NMF vs ICSI/NMF}}>0.05$

Double asterisks denote significant differences in normal forms using one-way ANOVA and Bonferroni post-hoc test

$P_{\text{ANOVA}}<0.0001$; $P_{\text{IVF/NMF vs ICSI/NMF}}<0.05$; $P_{\text{IVF/NMF vs ICSI/MF}}<0.01$; $P_{\text{ICSI/NMF vs ICSI/MF}}<0.01$

COS controlled ovarian stimulation

Although a high percentage of embryos appeared to be of good morphology in all three groups, pregnancy rates were low. Previous studies in Bologna poor responders have reported very low [39–42] or zero live birth rates [38]. This might be due to the fact that pregnancy and live birth rates are not only due to the compulsory single embryo transfer but in addition to

the known increased incidence of chromosomal abnormalities associated with advanced maternal age. It has been shown that approximately 72–76 % of oocytes [43] and 78 % of embryos [44] are aneuploid in women aged 40 years or older.

Furthermore, it cannot be excluded that high doses of gonadotrophins used in controlled ovarian stimulation protocols

Table 2 Embryological and pregnancy outcomes of poor responder patients with a single oocyte retrieved

	IVF/NMF N=101	ICSI/NMF N=50	ICSI/MF N=92
	% (n) (95 % CI)		
Proportion of mature (MII) oocytes ^a	80.2 (81) (71.3–86.9)	92 (46) (80.6–97.4)	78.3 (72) (68.7–85.6)
Proportion of 2PN oocytes	65.3 (66) (55.6–73.9)	66.0 (33) (52.1–77.6)	58.7 (54) (48.5–68.2)
Proportion of 3PN oocytes	7.9 (8) (3.9–15.1)	2 (1) (0.0–11.5)	2.2 (2) (0.1–8.1)
Proportion of 1PN oocytes	2.9 (3) (0.7–8.7)	2 (1) (0.0–11.5)	0.0 (0) (0.0–4.8)
Proportion of damaged oocytes	0.0 (0) (0.0–4.4)	8 (4) (2.7–19.4)*	6.5 (6) (2.8–13.8)*
Embryo formation rate	63.4 (64) (53.6–72.1)	60 (30) (46.2–72.4)	53.3 (49) (43.1–63.1)
Proportion of good quality embryos per obtained embryos ^b	54.7 (35) (42.6–66.3)	56.7 (17) (39.2–72.6)	57.1 (28) (43.3–70.0)
Proportion of patients with embryo transfer	63.4 (64) (53.6–72.1)	60.0 (30) (46.2–72.4)	53.3 (49) (43.1–63.1)
Proportion of Day 2 transfers	64.1 (41) (51.8–74.7)	63.3 (19) (45.5–78.2)	57.1 (28) (43.3–70.0)
Proportion of Day 3 transfers	35.9 (23) (15.6–31.9)	36.7 (11) (21.8–54.6)	42.9 (21) (30.0–56.7)
Implantation rate (%) mean±SD (95 % CI)	8.9±28.8 (1.2–16.6)	10±30.5 (–1.4–21.4)	8.2±27.7 (0.2–16.1)
Live birth rate	5.0 (5) (1.9–11.4)	4.0 (2) (0.34–14.2)	3.3 (3) (0.7–9.6)
Proportion of early pregnancy loss per positive hCG	61.5 (8/13) (35.4–82.4)	71.4 (5/7) (35.2–92.4)	50 (3/6) (18.8–81.2)

Asterisks denote significantly higher proportion of damages oocytes in ICSI-NMF and ICSI-MF compared to IVF-NMF using Bonferroni post-hoc test (p level at 0.017 following Bonferroni correction). $P_{\text{chi-square}}=0.022$; $P_{\text{IVF/NMF vs ICSI/NMF}}=0.011$; $P_{\text{IVF/NMF vs ICSI/MF}}=0.011$)

NMF non-male factor, MF male factor

^a In IVF cases, number of mature oocytes was calculated on Day 1 by the sum of 2PN, 3PN, 1PN and unfertilized MII oocytes

^b Good quality embryos: 4 cells, grades 1–2 on Day 2; 6–8 cells, grades 1–2 on Day 3

may lead to impaired endometrial receptivity [45], although in the present study the majority of women (>60 % in all groups) were treated using minimal gonadotrophin doses in a modified natural cycle.

In patients with very low numbers of oocytes retrieved and non-male factor infertility, conflicting data have been reported, with either significantly higher fertilization rates using ICSI as compared to IVF [32], or similar between the two methods [31]. In both of the above studies, clinical pregnancy, implantation rates, live birth rates, cycle cancellation and early pregnancy loss did not differ between the two fertilization methods [31, 32], suggesting that embryo quality does not seem to be affected by the method of fertilization, but rather by the intrinsic quality of the gametes themselves [46].

Regarding the long-term effects on the health of offspring, follow up of children born following ICSI versus conventional IVF has been reassuring [47], showing that the incidence of congenital malformations [48] and psychomotor development [49] were similar with both fertilization methods. Recently,

however, ICSI was shown to be associated with a higher risk of birth defects [50], raising new concerns over the long-term safety of the method, although it seems likely that the increased risk may be associated with the age of the woman and the presence of male infertility rather with the fertilization method per se.

The availability of only one “precious” oocyte, especially in poor responding patients, considerably increases the pressure on clinicians, embryologists and patients, in order to maximize chances of successful fertilization, and presents a clinical dilemma regarding the optimal fertilization method. In such cases, fertilization failure equals no embryo transfer.

The current study suggests that Bologna poor responders with a single oocyte retrieved have a poor prognosis. However, reproductive outcomes are similar using ICSI and conventional IVF, irrespective of the fertilization method used or the semen quality of the male partner. Therefore, the decision of which fertilization method to choose in the presence of only one oocyte should be based primarily on semen quality, in

combination with the patient's previous history. The design of a randomized controlled trial with a large patient population is necessary to confirm the present findings.

Acknowledgments The authors wish to thank Dr George Petsas and Dr Ioannis Zorzovilis for clinical work, Mrs K. Anagnostara for embryology work, and Mrs G. Stavropoulou for patient coordination.

Funding/competing interests The study was self-funded. No external funding was obtained. The authors declare that they have no competing interests.

Authors' contributions IAS conceived the study, performed acquisition, analysis and interpretation of data, writing and revision of the manuscript, and embryology work. EMK and GTL participated in the analysis and interpretation of data, writing and revision of the manuscript. BCT and RN participated in the interpretation of data and revision of the manuscript. TGL had the general supervision of the study, participated in study design, analysis and interpretation of data, writing and revision of the manuscript, and performed clinical work. All authors read and approved the final manuscript.

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