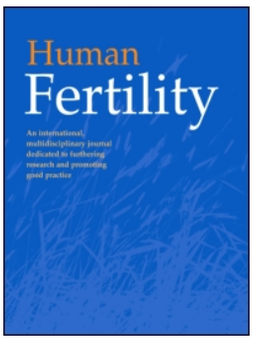

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ORIGINAL ARTICLE

Comparison of the assisted reproductive technology outcomes between conventional IVF and ICSI with donor oocytes in normozoospermic patients

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ABSTRACT

There is no evidence for the superiority of conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) using donor oocytes. This retrospective descriptive study aimed to compare the outcomes of conventional IVF ($n = 506$) and ICSI ($n = 613$) with donor oocytes in ($n = 968$) normozoospermic patients. Although the fertilization rate was statistically higher in the ICSI group ($p < 0.001$), conventional IVF provided better results than ICSI with respect to embryo quality (number of grade A embryos, $p < 0.001$). In addition, we observed more blastocysts in the conventional IVF group ($p < 0.001$) and more good quality embryos were obtained for cryopreservation compared to ICSI ($p < 0.001$). Regarding clinical results, there were no statistical significant differences in the positive pregnancy test, clinical pregnancy and clinical miscarriage rates between IVF and ICSI. However, the implantation rate was statistically higher when IVF was performed (50.4% vs. 43.0%, $p = 0.031$, OR (95% CI): 1.185 (1.050–2.530)). In conclusion, with the use of normozoospermic samples in our oocyte donation programme, IVF offers more embryo efficiency and increased implantation rates than ICSI.

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KEYWORDS
In vitro fertilization; ICSI; normozoospermic; embryo quality; embryo efficiency

Introduction

Conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) are the most frequently used techniques for achieving fertilization (Calhaz-Jorge et al., 2017; Cha, Oum, & Kim, 1997). Over the last few years, ICSI has become widely accepted technique for dealing with moderate or severe male factor infertility. Its use in Europe and the United States (USA) between 2002 and 2013 was greater than conventional IVF (Boulet et al., 2015; The European IVF-Monitoring Consortium (EIM), for the European Society of Human Reproduction and Embryology (ESHRE), Kupka et al., 2016). Decisions concerning the treatment choice (IVF or ICSI) are usually based on the assessment of male factor infertility (Shuai, Ye, Huang, & Xie, 2015), or on the outcome of previous IVF attempts. There are no widely accepted criteria, so decisions for couples with male subfertility are often empirical and may lead to complete fertilization failure after IVF, or to the unnecessary use of ICSI (Nardo, Granne, Stewart, & Policy & Practice Committee of the British Fertility Society, 2009; Plachot et al., 2002).

Recently, the application of ICSI has been greatly extended into the treatment of non-male factor infertility (NMFI) (Boulet et al., 2015). Bhattacharya et al. (2001) published the first prospective randomized trial comparing conventional IVF and ICSI in cases of NMFI showing that ICSI did not offer any advantage over IVF in terms of clinical outcome. The most recent Cochrane review which compares ICSI and conventional techniques concluded that whether ICSI should be preferred to IVF for cases of non-male factor subfertility remains an open question (van Rumste, Evers, & Farquhar, 2004). More recently, the use of ICSI with normal semen samples also showed no clinical benefit in certain groups of patients (Eftekhar, Mohammadian, Yousefnejad, Molaei, & Aflatoonian, 2012; Sfontouris et al., 2015; Tannus et al., 2017).

Major concerns have been raised over the implementation of unnecessary ICSI in the treatment of NMFI. The practice committee of the American Society for Reproductive Medicine concluded that there were no data to support the routine use of ICSI for NMFI (Practice Committees of the American Society for Reproductive Medicine & Society for Assisted Reproductive Technology, 2012). Although the main reason for using ICSI in NMFI is the fear of fertilization failure or low fertilization (Johnson, Sasson, Sammel, &

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Dokras, 2013), the real risk of failed fertilization is low and a similar frequency is found after using both IVF and ICSI (Practice Committees of the American Society for Reproductive Medicine & Society for Assisted Reproductive Technology, 2012). Moreover, the reproductive risks associated with ICSI in male factor infertility, such as increase of sex or autosomal chromosome aberrations, congenital anomalies, and imprinting disorders, are unknown in cases of NMFI (Evers, 2016; Practice Committee of American Society for Reproductive Medicine, 2008). Fertilization failure in some IVF cycles was prevented by ICSI, but the pregnancy outcomes were not improved (Lee, Lee, Park, Yang, & Lim, 2017).

It is difficult to compare the effect of the insemination method on the fertilization of oocytes and embryo quality in separate IVF or ICSI cycles because differences among infertile couples might influence the fertilization of oocytes and embryo development (Lee et al., 2017). In the current literature, we found no evidence for the superiority of conventional IVF versus ICSI using donor oocytes. Thus, this study aimed to compare the assisted reproductive technology (ART) outcomes following conventional IVF and ICSI with donor oocytes in normozoospermic patients.

Materials and methods

Patients and study design

This was a retrospective descriptive study carried out at a single centre for assisted reproduction (AR) between 2014 and 2016. This study was conducted in accordance with the Declaration of Helsinki. Ethical approval for the study was not required because the study was performed retrospectively based on medical files review.

A total of 968 couples who underwent 1119 cycles of AR treatment with donor oocytes and fresh embryo transfer were included. The treatment was performed using conventional IVF ($n = 506$) or ICSI ($n = 613$). The inclusion criteria for women were donor oocyte recipient women ($\geq$6 donor cumulus–oocytes complexes (COCs)) who underwent one or more cycles during the study period and had a normal uterine cavity prior to embryo transfer and an endometrial thickness of $\geq$7 mm. Couples who had had a previous total failure of fertilization or low fertilization rate ($<$50%) in previous cycles, had experienced repeated implantation failure or/and repeated miscarriages were excluded.

Only normozoospermic semen samples (World Health Organisation, 2010) with more or three million progressive motile spermatozoa/ml after sperm preparation were used to perform IVF or ICSI. Altered seminal parameters, or a recovery of less than three million progressive motile spermatozoa/ml after seminal processing on the day of oocyte collection, were used for ICSI only, and, therefore, did not enter the study.

The patients in the study had previously been informed of the possibility of performing one of the two insemination techniques (IVF or ICSI). They were not blinded as they were informed about the method of insemination on the day of oocyte collection or on the day of fertilization. The decision to perform ICSI or IVF was reported by the embryologists to the clinicians before the technique was performed, as per our usual protocol.

Ovarian stimulation and preparation of the endometrium

A controlled ovarian stimulation of oocyte donors was performed using antagonist short protocol. Gonadotropin hormones (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) were administered through subcutaneous daily injections from the first day of the cycle onwards. On the fifth day, or when follicles of $>$15 mm diameter were observed, the administration of the gonadotropin-releasing hormone (GnRH) antagonists was initiated in order to prevent premature LH-surge, and subsequent ovulation. When three or more follicles of $\geq$18 mm diameter were observed, a bolus of GnRH analogues was administered to trigger ovulation in the following 36 to 38 hours. The endometrial preparation in oocyte recipients was performed at the same time through the administration of oestrogen via transdermal patches or oral tablets. In order to obtain the best endometrial synchronization for a fresh embryo transfer, vaginal progesterone was administrated on the night of the oocyte retrieval of the donor.

Oocyte retrieval and semen preparation procedures

Follicular fluid was obtained by trans-vaginal ultrasound-guided puncture. Women were sedated and the follicular fluid was analysed directly in the *in vitro* fertilization laboratory where the COCs were collected. Once identified, the COCs were washed and transferred to fertilization medium (FM, Cook Medical, Limerick, Ireland) in four-well culture plates. They were then stored in an incubator until fertilization procedure was performed.

Semen samples were collected via masturbation on the same day as oocyte retrieval. Sperm preparation was performed by density gradients, as established for normozoospermic samples. Before sperm processing, sperm concentration and motility were assessed.

IVF procedure

The oocytes retrieved from the follicular fluid were placed on a four-well plate with FM and distributed according to quantity with up to four COCs to each well. Thirty microlitres of sperm sample, adjusted to a concentration of 150,000 sperm/ml were added to each well. After insemination, the oocytes were incubated at 37 °C and low oxygen pressures (5%) for 16 to 18 hours.

ICSI procedure

The oocytes were denuded from surrounding cumulus cells allowing to observe the progress of oocyte maturation prior to ICSI. After denudation, only mature metaphase II (MII) oocytes were microinjected. Once the ICSI technique (previously described by Ten, Mendiola, Vioque, de Juan & Bernabeu (2007)) had been completed, the microinjected oocytes were transferred to microdroplets (30μl) of FM until fertilization was confirmed.

Fertilization, embryo development and transfer procedures

Fertilization was confirmed 16 to 18 hours after insemination/microinjection, where two polar bodies (2PB) and two pronuclei (2PN) were observed. In order to assess fertilization rates, the COCs inseminated using IVF needed to undergo cumulus cell removal previously. Only in 53 cycles were embryos transferred on day 2/3 (short culture), with the rest (1066 cases) carried out on day 5/6 (long culture). For this, embryos were passed individually to 30 μl microdrops of fresh medium (CCM, Vitrolife. Göteborg, Sweden) on the morning of day 3. The embryos were evaluated on different days of development (2, 3, 5, 6) and were classified into four categories ranging from A to D according to the Spanish Association for Reproductive Biology embryo selection parameters (ASEBIR, 2015), previously described (Balaban et al., 2011). To avoid bias, embryo grading was performed by two senior embryologists. In addition, external quality controls organized by ASEBIR as well as internal controls were carried out for embryo assessment. High-quality embryos (grades A/B) were selected for embryo transfer and cryopreservation. Transfer was carried out using a catheter (Rocket Medical, USA) and ultrasound scan control.

Variables

Variables such as: (i) fertilization rate; (ii) implantation rate (number of gestational sacs visible on ultrasonography per embryo replaced, expressed as a percentage); (iii) chemical and clinical pregnancy rates; as well as (iv) chemical and clinical miscarriage rates, were recorded. Chemical pregnancy was defined by positive beta-hCG 14 days after embryos transfer. Clinical pregnancy was identified as observation of foetal heart activity by transvaginal ultrasonography.

Statistical analysis

The SPSS (20.0.0, Inc., Chicago, IL, USA) was used for data analysis. Categorical variables were analysed with the Pearson's chi-square test and continuous variables with the Student's t-test. Significance was defined as $p < 0.05$. Regarding clinical results, a logistic regression model adjusted to the confounders factors (oocyte recipient women age, number of donated COCs, number of inseminated or injected oocytes, fertilization rate, number of good quality embryos and number of transferred embryos) was performed.

Results

Table 1 shows the characteristics of participants and details of their treatment cycles. No statistically significant differences in the male age and oocyte donor age, days of follicular phase or endometrial thickness were observed. The number of donated COCs was statistically higher in the ICSI group compared to the IVF group (12.27 vs. 11.82 respectively; $p = 0.004$). However, due to the process of denudation, the number of mature injected oocytes was statistically lower

Table 1. Characteristics of study sample and details of treatment cycles in IVF and ICSI (mean ± SD).

	IVF ($n = 506$)	ICSI ($n = 613$)	p
Age of donor oocyte recipient women (years)	41.7 ± 4.1	40.8 ± 4.2	0.001
Male age (years)	41.36 ± 6.1	41.63 ± 5.9	0.470
Oocyte donor age (years)	24.66 ± 3.7	24.93 ± 3.7	0.210
Duration of follicular phase (days)	19.02 ± 3.1	18.93 ± 3.1	0.620
Endometrial thickness (mm)	8.00 ± 1.7	8.04 ± 1.7	0.720
No donated COCs	11.82 ± 2.3	12.27 ± 2.8	0.004
No inseminated/injected oocytes	11.82 ± 2.3	10.66 ± 2.5	<0.001

Table 2. Comparison of fertilization rate (%) and embryo quality between conventional IVF and ICSI on the day of transfer and blastocyst formation rate (mean ± SD).

	IVF	ICSI	p
Fertilization rate (%)	69.54	77.20	<0.001
Total number of 'Grade A' embryos	1.75 ± 1.6	1.30 ± 1.4	<0.001
Total number of 'Grade B' embryos	2.05 ± 1.5	1.89 ± 1.5	0.075
Total number of 'Grade C' embryos	0.43 ± 0.8	0.63 ± 1.0	<0.001
Total number of 'Grade D' embryos	1.49 ± 1.8	1.34 ± 1.9	0.202
Total number of blastocysts	4.94 ± 2.5	4.37 ± 2.3	<0.001

Table 3. Comparison of quality and number of embryos transferred and number of frozen embryos between conventional IVF and ICSI (mean ± SD).

	IVF	ICSI	p
Number transferred embryos ('Grade A')	1.05 ± 0.7	0.90 ± 0.8	0.001
Number transferred embryos ('Grade B')	0.48 ± 0.7	0.61 ± 0.7	0.03
Number transferred embryos ('Grade A + B')	1.53 ± 0.6	1.51 ± 0.6	0.553
Number transferred embryos ('Grade C')	0.06 ± 0.3	0.19 ± 0.5	<0.001
Number transferred embryos ('Grade D')	0.05 ± 0.4	0.02 ± 0.2	0.172
Number transferred embryos ('Grade C + D')	0.11 ± 0.5	0.21 ± 0.5	0.001
Total number of transferred embryos	1.60 ± 0.5	1.70 ± 0.5	<0.001
Total number of cryopreserved embryos	2.66 ± 2.3	2.19 ± 2.0	<0.001

than the number of inseminated COCs (10.66 vs. 11.82; $p < 0.001$) (Table 1).

The fertilization rate was affected statistically by the technique of insemination performed: 77.15% in ICSI and 69.54% in IVF ($p < 0.001$) (Table 2). Conversely, conventional IVF significantly increased the number of type A embryos of the entire cohort on the day of transfer in comparison to ICSI, ($p < 0.001$) (Table 2). Moreover, the blastocyst formation rate was statistically higher in conventional IVF compared to ICSI ($p < 0.001$), as was the case of the number of cryopreserved embryos ($p < 0.001$) (Tables 2 and 3, respectively). Therefore, usable embryos (both transferred and cryopreserved embryos) were greater when conventional IVF was used.

In terms of the quality of transferred embryos, more grade A embryos were transferred in the IVF group than in the ICSI group (1.05 vs. 0.90, respectively; $p = 0.001$) and more grade B embryos were transferred in the ICSI group than in the IVF group (0.61 vs. 0.48, respectively, $p = 0.003$). However, when the 'good quality' (grades A and B) embryos were grouped together, no significant differences between the two groups ($p = 0.553$) were observed (Table 3). In addition, more 'poor quality' (grades C and D) embryos were transferred in the ICSI group and statistically significant differences were observed ($p = 0.001$) between the two groups (Table 3).

There was a statistically significant difference between ICSI and IVF with regard to the number of embryos transferred (1.7 vs. 1.6, respectively; $p < 0.001$) (Table 3). Thus, this variable was considered as a confounding factor in the logistic regression analysis to extract clinical data. Most of the embryos were transferred during the blastocyst stage (day 5/6 of embryo development) and no significant differences were observed between the groups in terms of the day on which the transfer was performed based on the culture type (long or short) (Table 4).

Table 4. Comparative on day of transfer between IVF and ICSI.

	IVF	ICSI	p
Long-term culture ($n = 1066$)	587	479	0.400
Short-term culture ($n = 53$)	26	27	0.400

Table 5. Comparison of IVF and ICSI ART outcomes.

	IVF	ICSI	p*	OR (95% CI)*
Chemical pregnancy (Beta-hCG) rate (%)	67.9	63.0	0.291	1.177 (0.870–1.592)
Biochemical miscarriage rate (%)	12.5	11.5	0.731	1.080 (0.696–1.677)
Clinical pregnancy rate (%)	55.0	51.1	0.404	1.120 (0.840–1.492)
Multiple pregnancy rate (%)	27.5	25.5	0.475	1.176 (0.754–1.836
Clinical miscarriage rate (%)	14.2	11.9	0.949	0.981 (0.538–1.787)
Implantation rate (%)	50.4	43.0	0.031	1.185 (1.050–2.530)

*Adjusted by oocyte recipient women age, number of donated COCs, number of inseminated/injected oocytes, fertilization rate, number of good quality embryos (A + B) and number of transferred embryos.

Table 5 shows the comparison of outcomes following IVF and ICSI. In the IVF group, the implantation rate was significantly higher than in the ICSI group (50.4% vs. 43.0%, respectively; $p = 0.031$). There were no significant statistical differences in terms of the chemical, clinical and multiple pregnancy results, as well as biochemical and clinical miscarriage results (Table 5).

Discussion

Major concerns have been raised over unnecessary implementation of ICSI in the treatment of NMFI (Practice Committees of the American Society for Reproductive Medicine & Society for Assisted Reproductive Technology, 2012). In addition, we did not find any studies comparing ICSI and conventional IVF methods in an oocyte donation programme. Therefore, this study examined the convenience of using conventional IVF or ICSI in an egg donation programme using normal semen samples. Our findings show a significant increase in the fertilization rate in the ICSI group. However, the embryo quality, the number of usable embryos (transferred and cryopreserved) and the implantation rate were higher following conventional IVF. On the other hand, no significant difference was observed in miscarriage rate and clinical pregnancy rate between both groups.

Following the recommendations of the Vienna consensus for the calculation of the fertilization rate, the number of mature oocytes (MII) after cumulus cell removal were considered for ICSI and the number of inseminated COCs were used for conventional IVF (ESHRE Special Interest Group of Embryology & Alpha Scientists in Reproductive Medicine, 2017). Therefore, immature oocytes (GV and MI) observed during the evaluation of fertilization were taken into account in the case of IVF. The significant increase in the fertilization rate after ICSI compared to IVF is in agreement with other previously published studies where normozoospermic samples (Kim et al., 2014) and samples with moderate oligoasthenozoospermia (Xie, Zhu, & Huang, 2013) were used. In the case of the latter, the oligoasthenozoospermia factor could lead to fertilization failure following IVF due to its relationship with poor motility, abnormalities in the sperm head and, in 50% of cases, DNA fragmentation (Dorado et al., 2008; Flores, Lobo, & Chelhod, 2012). On the contrary, when normozoospermic samples were used and an advanced maternal age factor was present, no significant differences in terms of the percentage of fertilization after carrying out ICSI compared to IVF were observed (Tannus et al., 2017). However, other authors found differences in the fertilization rate in favour of IVF when samples with non-male factor were used (Eftekhar et al., 2012), although the rates are well below those recently published in previous studies and reported in this research. It is worth mentioning that in our study, when we considered the number of fertilized oocytes (2PN + 2PB) with respect to the total number of COCs donated, the fertilization rate was higher in the conventional IVF group compared to the ICSI group (69.3 vs. 66.0%, respectively) without statistical significance ($p = 0.07$, data not shown in the tables). This is probably due to the meiotic progression of immature eggs and late fertilization event after conventional insemination. The 'real' fertilization rate shown in Table 2 was considered as a confounding factor and taken into consideration in the logistic regression analysis to extract the clinical outcomes.

Regarding the IVF laboratory processes, and due to the variability that may exist based on the experience of the practitioners (Paternot et al., 2011), it is important to note that during the period of study, four clinical embryologists with more than 10 years of experience were responsible for performing microinjections, inseminations and embryo morphology assessment. There were no statistically significant differences between the fertilization rates obtained by the four embryologists who performed the microinjections and inseminations during this period (data not shown).

The fact that the number of grade A embryos was higher in IVF group than in ICSI group is related to the significant increase in the number of blastocysts (Table 2) and in the number of cryopreserved embryos (Table 3) obtained in the IVF group. Therefore, usable embryos increased when conventional IVF was used. Tannus et al. (2017) also found more usable embryos when normozoospermic semen samples were used including cases of advanced maternal age factor. Similarly, Wang et al. (2017) found that the blastocyst formation and embryo utilization rates in the ICSI group were significantly lower than those observed in the conventional IVF group. However, this could be influenced by the criteria used for ICSI in this study (abnormal semen parameters or previous fertilization failure with conventional IVF) (Wang et al., 2017).

An increase in the number of blastocysts and the number of cryopreserved embryos may represent an improvement in the optimization of the fertilization protocol in favour of conventional IVF with normal seminal parameters that may lead to an increase in the cumulative pregnancy rate. As donor oocytes were used in this study, there was no bias or differences due to female factors. As a possible hypothesis, this could be due to self-selection among competing sperm in conventional insemination which results in fertilization by higher quality sperm than when selected by an embryologist for ICSI. Alternatively, the intact cumulus may provide more nourishment for the zygote than the eggs stripped for ICSI.

In relation to the implantation rate, the increase observed in the IVF group compared to the ICSI group is consistent with the results of previous studies (Bhattacharya et al., 2001; Eftekhar et al., 2012; Xie et al., 2013). However, unlike Eftekhar et al. (2012) who found significant data in favour of the IVF, we did not find significant differences in pregnancy outcomes between groups.

Previous studies compared the effect of ICSI and IVF on sibling own oocytes, and they were not able to assess implantation or pregnancy outcomes (Chiamchanya, Tor-udom, & Gamnarai, 2008; Eftekhar et al., 2012; Komsky-Elbaz et al., 2013; Lee et al., 2017). However, the effectiveness of split insemination remains controversial. A recent publication shows that fertilization failure was prevented in some IVF cycles by ICSI, but the pregnancy outcomes were not improved (Lee et al., 2017).

Though the retrospective design of this study was a limitation, our findings are in agreement with the

Practice Committee of American Society for Reproductive Medicine (2008) and Evers (2016), and it puts in the spotlight the indiscriminate use of ICSI in the cases of NMFI. This is even more alarming in egg donation programmes, since there is a transition to using cryopreserved oocytes to facilitate the management of these programmes. Thus, ICSI should be prescribed in those cases in which there are semen parameters that may affect reproduction success (Fishel et al., 2000; Tucker, Wiker, & Massey, 1993) and also in cases of infertility with no apparent cause (Bungum, Bungum, Humaidan, & Andersen, 2004; Calderón et al., 1995; Ruiz et al., 1997).

In conclusion, although ICSI improved the fertilization rate, IVF increased the number of usable embryos and implantation rates compared to ICSI in the presence of normal sperm and donor oocytes. However, the method of insemination did not appear to influence the pregnancy and miscarriage rates. Thus, our findings demonstrate that the use of conventional IVF is the best option for couples undergoing oocyte donor treatment cycles with normozoospermic semen quality.

Disclosure statement

The authors report no conflict of interest.

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