

Successful Treatment of Severe Male Factor Infertility in Jamaica with Intracytoplasmic Sperm Injection

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ABSTRACT

Objective: To examine the impact of intracytoplasmic sperm injection (ICSI) on the treatment of subfertile couples in Jamaica.

Method: A review of the outcome of treatment cycles for infertile couples that underwent in-vitro fertilisation (IVF) and ICSI from 2003–05 at the Hugh Wynter Fertility Management Unit (HWFMU) of the University of the West Indies. Fertilisation and pregnancy rates for the cycles as well as the factors determining the success of the procedure were reviewed. SPSS 11.1 was used to do statistical calculations.

Results: Ninety-six ICSI cycles were done from January 1, 2003 to December 31, 2005. For couples with previous poor or no fertilisation in a standard IVF group ($n = 12$), the fertilisation rate was 72%; for those with substandard semen ($n = 73$), the fertilisation rate was 77.5%, for those with semen retrieved by surgical sperm method ($n = 11$), the fertilisation rate was 59%.

The resulting live births were 0%, 12.5% and 27.3% respectively. There was a statistically significant impact of age on pregnancy rates as the mean age of the females in the previously poor or no fertilisation in a standard IVF group (39.08 ± 5.14) was greater than those of the substandard semen group (35.93 ± 4.22) [$p = 0.023$] as well as the group with surgical sperm retrieval (32.82 ± 6.65) [$p = 0.019$].

Conclusion: With ICSI, the fertilisation and pregnancy rates in Jamaica are comparable to international rates regardless of the cause of infertility. However, the age of the female partner does have a significant impact on the pregnancy rate following ICSI.

Keywords: Intracytoplasmic sperm injection, in-vitro fertilisation, male factor infertility, Jamaica

Tratamiento Exitoso de la Infertilidad Severa por Factor Masculino en Jamaica Mediante Inyección de Esperma Intracitoplasmático

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RESUMEN

Objetivo: Examinar el impacto de la inyección de esperma intracitoplasmático (IEIC) en el tratamiento de las parejas subfértiles en Jamaica.

Método: Se realizó un examen del resultado de los ciclos de tratamiento para las parejas infértiles que recurrieron a la fertilización in vitro (FIV) y a la IEIC de 2003 a 2005 en la Unidad de Tratamiento de la Fertilidad Hugh Wynter del Hospital Universitario de West Indies (HWFMU). Se examinaron las tasas de fertilización y embarazos en todos los ciclos así como los factores que determinan el éxito del procedimiento. Se usó el programa SPSS para realizar los cálculos estadísticos.

Resultados: Se realizaron noventa y seis ciclos de IEIC del 1^{ero} de enero de 2003, al 31 de diciembre de 2005. Para parejas con ninguna o pobre fertilización en un grupo estándar de FIV ($n = 12$), la tasa de fertilización fue 72%; para aquellos con semen subestándar ($n = 73$), la tasa de fertilización fue 77.5%; para aquellos con semen recuperado mediante recuperación quirúrgica de esperma ($n = 11$), la tasa de fertilización fue 59%. Los nacimientos vivos resultantes fueron 0%, 12.5%, y 27.3% respectivamente. Hubo un impacto estadísticamente significativo de la edad sobre las tasas de

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embarazo, ya que la edad promedio de las hembras en la fertilización previamente pobre o sin fertilización en un grupo FIV estándar (39.08 ± 5.14) fue mayor para las del grupo de semen subestándar (35.93 ± 4.22) [$p = 0.023$] así como las del grupo con recuperación quirúrgica del espermatozoon (32.82 ± 6.65) [$p = 0.019$].

Conclusión: *Con el uso de IEIC, las tasas de fertilización y embarazo en Jamaica, son comparables a las tasas internacionales, independientemente de cual sea la causa de la infertilidad. Sin embargo, la edad de la pareja hembra no tiene un impacto significativo sobre la tasa de embarazo una vez aplicada la IEIC.*

Palabras claves: Inyección de espermatozoon intracitoplasmático, IEIC, FIV, infertilidad por factor masculino, Jamaica

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INTRODUCTION

Infertility is defined as the inability to conceive for more than a year despite having regular unprotected intercourse (1). It is estimated that 15% of all couples have difficulty conceiving naturally (2). Up to half of all infertile couples have a male factor as the underlying cause and as many as 2% of all men will exhibit suboptimal sperm parameters (3). In a society such as Jamaica, involuntary childlessness is associated with significant social stigma and has caused emotional trauma and relationship strain.

Males with sperm parameters below the World Health Organization (WHO) normal values are considered to have male factor infertility [MFI] (4). Male factor infertility may be one or a combination of low sperm concentration (oligospermia), poor sperm motility (asthenospermia) or abnormal morphology (teratospermia).

Artificial insemination (AI) is used to treat mild forms of MFI. However, *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) is commonly used for couples with more severe forms of MFI (5–7). Intracytoplasmic sperm injection involves the injection of a single spermatozoon directly into the ooplasm of a mature oocyte thereby bypassing all the preliminary steps of fertilisation.

The technique was first described by Palermo *et al* (8) in 1988 and since the first live birth reported in 1992, this technique has become the preferred method for treating severe male factor infertility. Since 2000, this revolutionary technique has been helping Jamaican couples that would otherwise be childless, experience the joys of parenthood.

SUBJECTS AND METHOD

This is a retrospective study consisting of couples presenting to the Hugh Wynter Fertility Management Unit (HWFMU), between January 1, 2003 and December 31, 2005. Couples were excluded if the male partner had normal semen parameters (WHO criteria), if the couples used donor or frozen oocytes, or if the cycles involved split IVF and ICSI procedures.

All couples underwent the long gonadotrophin-releasing hormone (GnRH) agonist protocol which com-

prised down regulation of the hypothalamic-pituitary-ovarian axis *via* subcutaneous (SC) injection of the gonadotrophin-releasing hormone (GnRH) agonist buserelin acetate (Superfact, Hoechst AB, Frankfurt, Main, Germany) from day 21 of the woman's menstrual cycle. Down regulation was confirmed by transvaginal ultrasound scan (TVUS) evidence of endometrial thickness less than 4 mm and a serum oestradiol level less than 100pg/ml. Menotrophin (Menogon, Ferring Pharmaceuticals Ltd, GmbH, Wittland, Kiel Germany), a human menopausal gonadotrophin (hMG), with dosages ranging from 150–450 units was then used to stimulate ovarian follicular development. Follicular development was monitored by TVUS every two–three days from day seven of stimulation until at least three follicles attained a diameter in excess of 16 mm. Ovulation was triggered 34–36 hours before the egg recovery by administering 10 000 IU of human chorionic gonadotrophin (hCG) (Choragon, Ferring Pharmaceuticals Ltd, GmbH, Wittland, Kiel Germany) and egg recovery was facilitated by transvaginal ultrasound guided follicle aspiration.

Luteal phase support was achieved by given progesterone pessaries 400 mg (Cyclogest® Cox Pharmaceuticals, Whiddon Valley, Barnstable EX328NS, UK) vaginally twice daily and oestradiol 2 mg orally every eight hours.

Sperm Preparation Method

On the same day as egg recovery, sperms in the form of an ejaculate, aspirate or biopsy were prepared. The ejaculate or aspirate was placed on a 40/80 (Nidacon) PureSperm gradient, centrifuged and then the resulting pellet of functional sperm was washed in Medicult IVF Universal medium and centrifuged again. The remaining pellet was incubated at 37°C with 5% carbon dioxide for approximately three hours. The biopsied sample would be macerated into Medicult Flushing medium and the resulting solution was then treated as an ejaculated sample.

Sperm criteria for ICSI

Couples were selected for ICSI based on the sperm parameters outlined in Table 1. Furthermore, couples that had

Table: Indications for ICSI at the HWFMU

Parameters	Values
Sperm concentration	≤ 6 million
Sperm motility	≤ 30%
Sperm morphology	≤ 4% normal
Poor fertilisation with previous standard IVF	≤ 20%
Men requiring surgical sperm retrieval (SSR)	all

ICSI = Intracytoplasmic sperm injection; HWFMU = Hugh Wynter Fertility Management unit; IVF = *in vitro* fertilisation

poor fertilisation ($\leq 20\%$) with previous standard IVF treatment or those requiring surgical sperm retrieval (SSR) for men with azoospermia or vasectomy were offered ICSI. There is currently no established international ICSI criteria standard, thus different units have varied parameters for using ICSI (4, 5).

The final decision for ICSI treatment was based on the results from the semen sample produced on the day of the egg recovery (ER). If the sperm parameters on the ER day were within normal ranges (WHO criteria) IVF was performed instead of ICSI.

ICSI procedure

Oocytes, recovered *via* TVUS guided aspiration, had their *corona cumulus* complex removed with the aid of Hyadase (SynVibro®) and mechanical manipulation using sterile glass pipettes. Mature oocytes (metaphase II) were selected and then transferred into an insemination dish and incubated at 37°C with 5% CO₂ in paraffin covered microdroplets.

Approximately 1 microlitre of the prepared sperm was placed in Medicult® polyvinylpyrrolidone (pvp) microdroplet to reduce the motility of the sperm and aspirated into the injection pipette. The mature oocyte was immobilized by a slight suction from the holding pipette, the injection pipette was introduced into the *zona pellucida* and the contents of the injection pipette gently expelled into the ooplasm (Fig. 1).



Figure: ICSI (the injection of a single sperm into an oocyte).

Injected oocytes were then incubated under laboratory conditions. Fertilisation of the injected oocytes was assessed 16–18 hours after the ICSI procedure as identified by the presence of two clearly distinct pronuclei.

Fertilisation and Embryo Transfer

Embryos that achieve normal fertilisation were incubated for a further 24–48 hrs. Cleavage of the embryos was an indicator of normal development. A maximum of three embryos was transferred into the womb 48–72 hours after the ICSI procedure *via* an embryo transfer catheter. The number transferred was determined by factors such as age of the female patients, aetiology of infertility, number of embryos available and embryo development.

A urine pregnancy (hCG) test was done two weeks after the embryo transfer to determine pregnancy. Only clinical pregnancies, as defined by ultrasound evidence of live intrauterine pregnancy at six weeks of gestation, were included in the study.

This study aims to determine the success of ICSI for male factor infertility and to assess the use and outcomes of ICSI for non-male factor infertility at the Hugh Wynter Fertility Management Unit (HWFMU) of the University of the West Indies.

RESULTS

A total of 96 ICSI – transfer cycles were conducted from January 1, 2003 to December 31, 2005. Over this time period, 969 oocytes were collected, 707 were injected with a single sperm from semen samples. All IVF-ICSI cycles resulted in fertilisation of mature metaphase II oocytes and had embryos transfer two-three days later. The mean number of embryos transferred per ICSI cycle was 2.36 ± 0.70 .

The number of mature eggs obtained per cycle as well as the fertilisation rates obtained were not significantly affected by how semen was collected *ie* ejaculate or SSR, or whether the patient had previously failed fertilisation in a standard IVF or not ($p = 0.44$ and 0.07 respectively). For couples with previous poor or no fertilisation in a standard IVF cycle, the fertilisation rate was 72% ($n = 46$); for those with substandard semen, the fertilisation rate was 77.5% ($n = 435$), for those with semen retrieved by SSR the fertilisation rate was 59% ($n = 48$).

The clinical pregnancy rate for couples with a previously poor fertilisation in a standard IVF cycle was 0%; for those with substandard semen, the clinical pregnancy rate was 26% ($n = 19$) and for those with semen retrieved by SSR, the clinical pregnancy rate was 36.4% ($n = 4$). The resulting live birth rates were 0%, 12.5% ($n = 9$), and 27.4% ($n = 3$) respectively. Age incurred a statistically significant impact on pregnancy rates as the mean age of the females in the previously poor or no fertilisation in a standard IVF group (39.08 ± 5.14) was greater than those of the substandard semen group (35.93 ± 4.22) [$p = 0.023$] as well as the group with SSR (32.82 ± 6.65) [$p = 0.019$].

A total of 23 clinical pregnancies resulted from the 96 ICSI transfers: 22 singletons and one set of twins. The overall pregnancy rate (PR) was 24% ($n = 25$). There were 13 live births comprising 11 singletons and 1 set of twins giving an overall live birth rate of 13.5% ($n = 13$).

To ascertain whether significant differences existed between the mean number of mature eggs obtained, number of eggs fertilised and female partner's age, an ANOVA test of variance was done which showed that the female partner's age was significant ($p = 0.007$). The age of the female partner for the previously failed fertilisation in a standard IVF group was significantly higher than those of the poor semen group and the SSR group, $p = 0.019$ and 0.023 respectively.

DISCUSSION

The main criterion for the use of ICSI is the presence of at least one viable sperm for every oocyte injected (10). The main condition that has the potential to affect ICSI success rates is the use of dead sperm (11). Even in the most extreme case of male subfertility, ICSI can fertilise an egg and result in a pregnancy. The type or degree of sperm impairment has little or no importance on the outcome of ICSI (10).

The ICSI fertilisation rates in this series for suboptimal sperm obtained from an ejaculate and SSR samples (77.5% and 59%) are higher than those quoted by Plachot *et al* (4) in 2002 and Van Steirteghem *et al* (12) in 1993. However, the rates in this study are within the range (50–80%) quoted by the Human Fertilisation and Embryology Authority [HFEA] (13) and the American Society of Reproductive Medicine (ASRM) (14). Silber *et al* (15) in 1995 used SSR samples from the epididymis and the testis and reported fertilisation rates of 45% and 46% respectively. In Sibley's series, for patients with severe oligoasthenoteratospermia, the fertilisation and pregnancy rates were 79% and 25% respectively. In patients with obstructive azoospermia, for whom epididymal spermatozoa were used, these were 75% and 28%, and in the non-obstructive group for which testicular spermatozoa were used for injection, they were 69% and 21% respectively. These rates were not significantly different in the two groups ($p = 0.85$ and $p = 0.14$, respectively).

The number of mature eggs obtained per cycle as well as the fertilisation rates obtained were not significantly affected by the source of semen, *ie* ejaculate or SSR, or whether the patient had previously failed fertilisation in a standard IVF or not ($p = 0.44$ and 0.07 , respectively). These results are in keeping with the findings of other authors that semen characteristics obtained from males have little or no influence on fertilisation rate (16, 17).

Sibley and Nagy (1997) reported that other factors such as female age and ovarian reserve negatively influence ICSI outcomes (18). In this series, the age of the female partner for the previously failed fertilisation in a standard IVF group was significantly higher than those of the poor semen group and the SSR group $p = 0.019$ and 0.023 , respectively. Since semen characteristics were not significant factors, this

implies that oocyte quality is the likely factor in the poor outcomes for those who previously had failed fertilisation in a standard IVF. For the period reported, donor eggs were not used at the unit; however, its introduction since early 2007 has begun to ameliorate these results and is a subject of review currently.

Matorres *et al* (17) prospectively followed 285 couples with severe male factor infertility that were awaiting ART for two years. The per month spontaneous pregnancy rates were 0.13% overall, 0.32% for non-azoospermic men and 0% for azoospermic men. Of note, however, regardless of the source of the sperm used for ICSI cycles, fertilisation, cleavage, pregnancy and live births can result. The fact that no pregnancies occurred in the group with previously failed fertilisation in a standard IVF underscores the fact that other factors seriously impact on the success of ICSI.

The application of ICSI does present challenges and concerns. Clearly, the disruption of the oocyte's plasma membrane is an invasive act raising the question of sufficient injury to the oocyte resulting in its demise. Approximately, 7–14% of the metaphase II oocytes will die after ICSI (18). For the embryos which survive, questions arise concerning the effect of mechanical disruption of the oocyte resulting in potential birth defects not seen in natural fertilisation or conventional IVF.

Of concern to both parents and IVF specialists is the possible genetic impact that the use of sperm from men suffering from severe male infertility will have on the resulting offspring. Studies have concluded that 13% of azoospermic men and 7% of severely oligospermic men will carry the Y chromosome deletion [AZFc deletion] (3, 4, 19). Intracytoplasmic sperm injection treatment of Y chromosome deletion in infertile couples risked transmitting this defect to any resulting son, who will in turn encounter a similar male infertility problem in the future (20). These genetic alterations are more likely related to the underlying genetic causes of oligospermia than the actual ICSI technique.

In Jamaica, children resulting from ICSI treatment are still too young for any substantial investigation to be carried out. Although world data is not conclusive on the long term outcome of ICSI compared with naturally conceived children, neither neurodevelopmental scores nor rates of malformation were negatively affected by ICSI in large scale studies in the United Kingdom (21) and Sweden (22) respectively. Further studies and investigations still need to be done for any conclusive statement to be made regarding the genetic impact that ICSI may have on the resulting offspring in Jamaica.

In summary, ICSI at the Hugh Winter Fertility unit at the University of the West Indies, has allowed for fertilisation and pregnancy in patients who would otherwise have had minimal or no chance of childbearing. Success rates obtained in this unit are in keeping with international data. Intracytoplasmic sperm injection is best applied to male factor infertility. However, the age of the female partner does

have a significant impact on the pregnancy rate following ICSI.

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