Effectiveness of in vitro fertilization with intracytoplasmic sperm injection for severe male infertility

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Abstract

Background: In its 1993 report the Canadian Royal Commission on New Reproductive Technologies challenged the effectiveness of in vitro fertilization for severe male infertility. To address the Commission's concern, the authors compared the relative effectiveness of in vitro fertilization combined with intracytoplasmic sperm injection for severe male infertility and conventional in vitro fertilization for complete tubal occlusion in women.

Methods: This historical cohort study was done at the PROCREA Fertility Centre, a private tertiary human reproduction centre in Montreal. Three groups of infertile couples were compared: 122 couples with severe male infertility treated by in vitro fertilization with intracytoplasmic injection of fresh sperm from ejaculate (group 1); 27 couples with obstructive azoospermia treated by in vitro fertilization with intracytoplasmic injection of epididymal sperm (collected by microepididymal or percutaneous epididymal sperm aspiration) (group 2); and 98 couples with tubal factor infertility (bilateral tubal occlusion) treated with conventional in vitro fertilization (with sperm from ejaculate) (group 3). The main outcomes measured were rates of fertilization, pregnancy, clinical pregnancy and implantation.

Results: Pregnancy rates per started cycle were 35%, 40% and 34% for groups 1, 2 and 3 respectively. When prognostic factors were controlled for, none of the outcome measures differed significantly between the 3 groups.

Interpretation: In vitro fertilization with intracytoplasmic injection of sperm from the ejaculate or the epididymis is as effective for treating severe male infertility as conventional in vitro fertilization is for treating complete occlusion of the fallopian tubes in women.

In vitro fertilization is the last option for couples with infertility who have tried and failed to conceive using standard therapies such as surgery, fertility drugs and artificial insemination. It was successfully applied for the first time in 1978 to bypass complete occlusion of fallopian tubes.[1] Since then, the indications for in vitro fertilization have expanded internationally to include other infertility conditions such as male infertility, endometriosis-associated infertility, immunological infertility and unexplained infertility.[2,3] In Canada the efficacy of in vitro fertilization has so far been recognized by governmental authorities only for tubal blockage, which accounts for approximately 45% of in vitro fertilization treatments. In fact, the efficacy of in vitro fertilization for other so-called "subfertility" indications, such as male infertility, was challenged by the Royal Commission on New Reproductive Technologies.[4] In its report, *Proceed with Care*, the Royal Commission recognized complete occlusion of fallopian tubes as an indication for in vitro fertilization. However, it was reluctant to endorse this method for couples with other causes of infertility if at least one of the woman's fallopian tubes was permeable, because it felt that the technique had not yet been rigorously evaluated for these other forms of infertility. More specifically, the Commission recommended that:

> 128. IVF for bilateral fallopian tube blockage be an insured service under provincial medicare programs within the regulatory framework recommended by the Royal Commission on New Reproductive Technologies

Evidence

Études

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129. The province of Ontario discontinue coverage of IVF [in vitro fertilization] for indications other than bilateral fallopian tube blockage and that the resources now devoted to those services be reallocated to fund clinical trials of unproven but promising techniques.

Unfortunately, the Royal Commission's recommendations have had and are still having an unfavourable impact on the access of infertile Canadian couples to medical treatment. For example, on the basis of the Royal Commission's recommendations, the Ontario government has restricted coverage for reproductive technologies to women with complete obstruction of the fallopian tube.

Since the publication of the Royal Commission report, in vitro fertilization with intracytoplasmic sperm injection has been introduced as a potential treatment for severe male infertility, and high rates of fertilization and pregnancy have been reported.[5,6] In the absence of spermatozoa in the ejaculate (azoospermia), the introduction of microepididymal and percutaneous epididymal sperm aspiration followed by intracytoplasmic injection of sperm also appeared to be potential breakthroughs for the treatment of severe male infertility.[7]

The objective of this study was to address some of the Royal Commission's concerns by comparing the effectiveness of in vitro fertilization (with intracytoplasmic injection of sperm from the ejaculate or the epididymis) for severe male infertility with that of conventional in vitro fertilization for complete tubal occlusion in women.

Methods

Medical records were reviewed and data extracted for 3 groups of infertile couples who underwent treatment at the PROCREA Fertility Centre, a private tertiary human reproduction centre in Montreal, between January 1996 and January 1998: 122 couples with severe male-factor infertility (severe oligo-astheno-teratozoospermia) who were treated with intracytoplasmic injection of ejaculated fresh sperm (group 1); 27 couples with obstructive azoospermia who were treated with intracytoplasmic injection of epididymal sperm; and 98 couples with tubal-factor infertility (bilateral tubal occlusion) who were treated with conventional in vitro fertilization (group 3). In total, 247 infertile couples were included in the study.

The eligibility and selection criteria for this historical cohort study were as follows: couples who had used donor sperm or oocytes were excluded, and for each couple, only one treatment procedure was included (for couples for whom there was more than one treatment procedure during the study period, data from the last such procedure were analyzed). Male-factor infertility was diagnosed on the basis of 1992 World Health Organization criteria.[8] All infertile couples who were seen at the PROCREA Fertility Centre during the study period and who met the eligibility criteria were included.

For the 27 couples with obstructive azoospermia (group 2), 22 of the treatment cycles involved percutaneous epididymal sperm aspiration and 5 involved microepididymal sperm aspiration. The latter procedure was used only if percutaneous epididymal sperm aspiration failed to yield any sperm. The causes of azoospermia among these 27 men were failed vasovasostomy (15 patients), bilateral congenital absence of the vas deferens (6), vasectomy (4), obstructive azoospermia caused by epididymal infection (1 patient) and bilateral agenesis of the seminal vesicles (1 patient). Because of the previously established association between congenital absence of the vas deferens and cystic fibrosis,[9] these men were screened for mutations before treatment was undertaken.

All patients provided informed consent before treatment was initiated. Institutional ethics approval was obtained for examining patients' medical charts for the purposes of this study.

For ovarian stimulation, a long standard protocol, described previously,[10] was used. In brief, leuprolide, a gonadotropin-releasing hormone agonist, was started on the first day of the menstrual cycle. Two weeks later, ovarian activity was determined by measuring serum levels of estradiol. If down-regulation was observed (i.e., estradiol less than 200 pmol/L), human menopausal gonadotropins were administered for approximately 10 days, with monitoring of follicular development (by ultrasonography) and serum estradiol levels. Administration of human menopausal gonadotropins was stopped when all of the following criteria had been met: the mean diameter of the largest follicle had reached 18 mm, at least one other follicle with a mean diameter of 16 mm was present and serum estradiol level was judged to be within the acceptable range for the number of mature follicles present. Human chorionic gonadotropin was then administered, on the day after the last injection of human menopausal gonadotropins.

Percutaneous or microepididymal sperm aspiration was per-

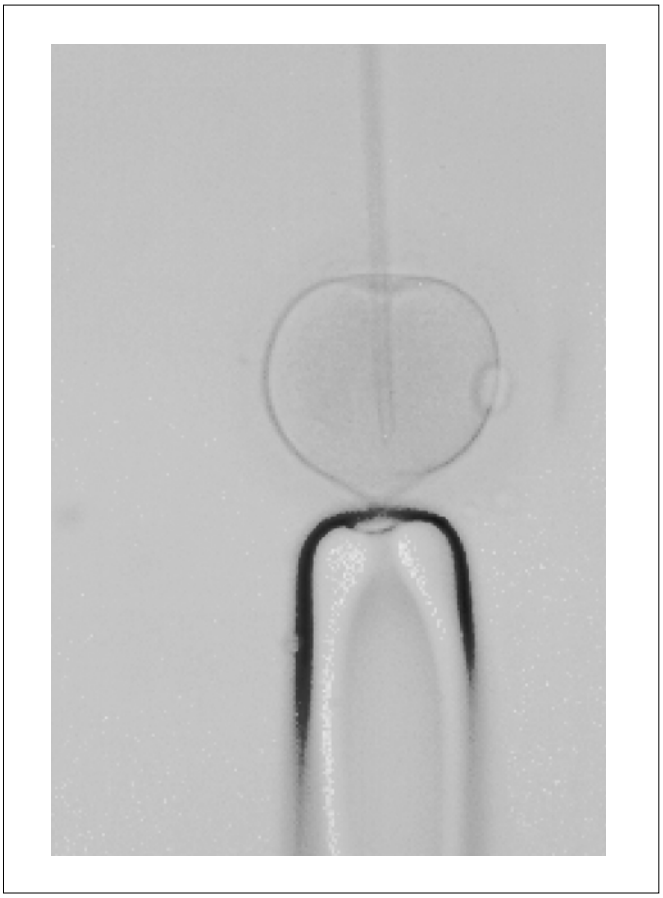

Fig. 1: Intracytoplasmic injection of sperm.

formed on the same day as collection of the female partner's eggs. Sperm aspiration was done with local anesthesia without intravenous sedation. For the percutaneous procedure, a tiny butterfly needle (no. 25) connected to a tuberculin syringe was inserted through the scrotal skin directly into the epididymis, and sperm were aspirated. For the microepididymal procedure, a small incision was made into the scrotum to expose the epididymis. With the aid of magnification, a single epididymal duct was opened, and sperm were aspirated through a fine needle.

Oocytes were recovered under vaginal ultrasound guidance 34 to 36 hours after administration of the human chorionic gonadotropin. For treatments involving intracytoplasmic sperm injection, laboratory fertilization was made easier through the microinjection of a single sperm inside an ovum (Fig. 1), thereby bypassing natural barriers such as the zona pellucida and the ooplasm membrane.

Intracytoplasmic injection of sperm was used for couples in groups 1 and 2. The cumulus of the oocyte was first removed with hyaluronidase. Only metaphase II oocytes underwent injection. The sperm injection was carried out with an inverted microscope equipped with 2 remotely controlled micromanipulators for positioning. Each spermatozoon was first immobilized, then aspirated, tail first, into the microinjection pipette. Each metaphase II oocyte was held by the holding pipette, with the polar body at the 12 o'clock or 6 o'clock position. The microinjection pipette was pushed through the zona pellucida into the ooplasma at the 3 o'clock position, and a single spermatozoon was injected. After the procedure, the oocytes were incubated in Upgrade B2 INRA medium (Laboratoire C.C.D., Paris, France) supplemented with 10% maternal serum.

For conventional in vitro fertilization (group 3), each oocyte was inseminated with approximately 100 000 spermatozoa 1 hour after eggs were collected.

The eggs were assessed for the first signs of fertilization (the presence of pronuclei and polar bodies) approximately 18 hours after injection of sperm or insemination. Embryo cleavage was evaluated 24 hours later, before embryo transfer. A maximum of 3 embryos were placed in the uterus 48 hours after recovery of the oocytes. Luteal support with vaginal micronized progesterone was started on the evening of embryo transfer.

Pregnancy was confirmed 2 weeks later with a quantitative estimation of serum β human chorionic gonadotropin. If the test result was positive, transvaginal ultrasonography was performed 2 weeks later to confirm the presence or absence of clinical pregnancy (fetal heart beat). The implantation rate was defined as the number of clinical pregnancies (presence of fetal heart beat detected by transvaginal ultrasonography at the 6th week of gestation) divided by the number of embryos transferred. This rate reflects the successful attachment of an embryo to the endometrium.

Comparisons of demographic, clinical and reproductive characteristics between the 3 groups were based on analyses of variance (with Scheffe's contrasts when necessary) for the quantitative characteristics and on Pearson's χ^2 for qualitative (or categorical) characteristics.

Effectiveness of the procedures was compared in 2 steps. First, crude comparisons were done without control of prognostic (or confounding) factors. Second, adjusted comparisons were done by means of multiple stepwise linear and logistic regressions.

Results

The demographic, clinical and reproductive characteristics of the 3 groups of couples are summarized in Table 1. There was a statistically significant difference ($p = 0.02$) among the groups in terms of the duration of infertility (the period for which couples had been trying to conceive before undergoing treatment): couples in group 2 (treated by intracytoplasmic injection of epididymal sperm) had a shorter period of infertility than the other 2 groups. There was also a statistically significant difference between the groups in terms of the type of infertility (primary or secondary), with a predominance of secondary infertility in group 3 couples (who had tubal-factor infertility treated with conventional in vitro fertilization) ($p < 0.001$). There was no significant difference among the 3 groups of patients with regard to age; mean number of oocytes retrieved, injected or fertilized; or mean number of embryos obtained or transferred (Table 1).

Table 1: Comparison of clinical and reproductive characteristics between the 3 groups of infertile patients

Characteristic	Group 1 ICSI with sperm from ejaculate *n = 122*	Group 2 ICSI with epididymal sperm *n = 27*	Group 3 Conventional IVF *n = 98*	*p* value*
Mean age (and SD), yr	34.1 (4.3)	32.4 (4.2)	34.5 (4.1)	0.08
% > 35 yr	50.8	25.9	51.0	
Mean duration of infertility (and SD), yr	4.9 (3.1)	3.0 (2.2)	4.3 (2.7)	0.02
% > 5 years	42.6	14.8	36.7	
Type of infertility, %				
Primary	67.2	55.6	37.5	< 0.001
Secondary	32.8	44.4	62.5	
Mean no. of oocytes retrieved (and SD)	10.5 (6.1)	11.0 (5.8)	8.9 (5.1)	0.08
Mean no. of oocytes injected or fertilized (and SD)	8.7 (5.0)	9.4 (5.2)	8.9 (5.1)	0.78
Mean no. of embryos (and SD)	4.9 (3.2)	5.6 (3.7)	5.8 (3.8)	0.14
Mean no. of embryos transferred (and SD)	2.5 (0.8)	2.7 (0.7)	2.6 (0.7)	0.49

Note: IVF = in vitro fertilization, ICSI = intracytoplasmic sperm injection, SD = standard deviation.
*p values are from analyses of variance (comparisons of means) or from Pearson's χ^2 (comparison of percentages).

The mean rate of fertilization was higher for couples with tubal-factor infertility who underwent conventional in vitro fertilization (67.2%) than for couples treated with intracytoplasmic injection of ejaculated sperm (57.9%) or epididymal sperm (58.0%). The rates of implantation, pregnancy and clinical pregnancy were similar for the 3 groups (Table 2).

Table 3 presents the significant predictors of fertilization, implantation, pregnancy and clinical pregnancy as determined by stepwise linear and logistic regression. Although the crude results indicated that fertilization rates were higher for those who underwent conventional in vitro fertilization (group 3), adjustment for other factors abolished the between-group differences. The only significant predictors of fertilization were the number of oocytes injected or fertilized and the number of embryos created (Table 3). There were no differences between groups for implantation rates, pregnancies per cycle and clinical pregnancies per cycle before or after adjustment for other factors.

Interpretation

In vitro fertilization was developed as a treatment for couples with infertility caused by complete occlusion of the fallopian tubes. In the 1980s, new indications for in vitro fertilization arose, including endometriosis-associated infertility, male-factor infertility and unexplained infertility. However, success rates were poor for severe forms of male infertility, particularly for those with less than 10^6 motile spermatozoa/mL. The report of the first pregnancy with intracytoplasmic injection of sperm[5] and the high fertilization rate reported by van Steirteghem and colleagues[6] brought new hope for couples with severe male-factor infertility.

In this study, the effectiveness of intracytoplasmic injection of sperm from the ejaculate or the epididymis for severe male infertility was compared with the effectiveness of conventional in vitro fertilization for complete obstruction of the fallopian tubes. No clinically significant difference was observed between the 3 groups in terms of the number of oocytes collected, the number of oocytes injected and fertilized, the number of embryos obtained and the number embryos transferred. The rates of fertilization, implantation, pregnancy and clinical pregnancy were similar among the 3 groups, which demonstrates that intracytoplasmic injection of ejaculated or epididymal spermatozoa is as effective for severe male infertility as conventional in vitro fertilization is for tubal-factor infertility.

The mean duration of infertility was 4.9 years for couples with severe male-factor infertility, 3.0 years for those with obstructive azoospermia and 4.3 years for those with tubal-factor infertility. This difference is not surprising, because couples in which the man has azoospermia have only a few alternatives, such as adoption, donor insemination and intracytoplasmic injection of sperm.

The duration of infertility is an important and powerful factor in determining the chance of pregnancy, with or without treatment. In this study the probability of conceiving with no therapy in cases of total sterility such as azoospermia (group 2) and bilateral tubal obstruction (group 3) was zero. For men with severe oligo-astheno-teratozoospermia (group 1), for whom the mean duration of infertility in this study was 4.9 years, the monthly probabil-

Table 2: Comparison of crude rates of effectiveness between the 3 groups of infertile patients

Measure of effectiveness	Group 1 ICSI with sperm from ejaculate *n = 122*	Group 2 ICSI with epididymal sperm *n = 27*	Group 3 Conventional IVF *n = 98*	*p* value
Mean fertilization rate (and SD)	57.9 (22.7)	58.0 (18.2)	67.2 (23.0)	0.01
Mean implantation rate (and SD)	11.0 (19.8)	10.2 (16.4)	10.3 (16.7)	0.95
Pregnancy rate/cycle, %	35.2	40.7	33.7	0.79
Clinical pregnancy rate/cycle, %	27.9	29.6	28.6	0.98

Table 3: Predictors of fertilization, implantation, pregnancy per cycle and clinical pregnancy per cycle

	Linear regression		Logistic regression	
Prognostic factors	Fertilization rate, mean difference (and 95% CI)	Implantation rate, mean difference (and 95% CI)	Pregnancy per cycle, odds ratio (and 95% CI)	Clinical pregnancy per cycle, odds ratio (and 95% CI)
Age, yr	–	–0.65 (–1.19 to –0.11)	–	–
No. of oocytes injected or fertilized	–0.52 (–0.48 to –0.56)	–	–	–
No. of embryos	8.6 (7.8 to 9.4)	–	–	–
No. of embryos transferred	–	–	2.08 (1.34 to 3.22)	2.14 (1.32 to 3.46)

Note: CI = confidence interval.

ity of pregnancy in the absence of treatment has been estimated at less than 1% on the basis of mathematical models.[11] Given such a low level of fecundity without treatment, recourse to methods of assisted conception should be supported.

We observed a negative association between age and rate of implantation (mean difference in implantation rate –0.65% per year of age [Table 3]). This inverse relation was observed within each of the 3 groups. A similar phenomenon has been described previously: age was found by van Kooij and colleagues[12] to be negatively associated with fertilization rates. The finding of a higher fertilization rate in the group with tubal-factor infertility was also expected; however, this difference disappeared when other factors were accounted for (Table 3).

Our study demonstrates that intracytoplasmic microinjection of sperm for severe male infertility has the same success rate as conventional in vitro fertilization for complete tubal occlusion in women. Couples who previously would have been offered donor insemination or adoption can now achieve pregnancy despite severe impairments in semen quality with procedures such as intracytoplasmic sperm injection, accompanied when necessary by microepididymal and percutaneous sperm aspiration. These procedures are minimally invasive, simple and repeatable. We believe that in vitro fertilization with intracytoplasmic injection of sperm should be offered as an effective treatment for severe male infertility. In addition, we suggest that recommendations 128 and 129 of the Royal Commission on New Reproductive Technologies be revised. The effectiveness of in vitro fertilization for severe male infertility, as demonstrated in this study, is sufficient to warrant provincial funding. Even though Ontario is the only province providing health care coverage of in vitro fertilization, it is discriminatory for Ontario or any other Canadian provinces to limit accessibility to advanced reproductive technologies for both men and women with other, equally treatable fertility problems. Canadian provinces need to reconsider their coverage of fertility treatments.

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