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Original article

An ICSI rate of 90% minimizes complete failed fertilization and provides satisfactory implantation rates without elevating fetal abnormalities

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ABSTRACT

IVF cycles utilizing the ICSI technique for fertilization have been rising over the 25 years since its introduction, with indications now extending beyond male factor infertility. We have performed ICSI for 87% of cases compared with the ANZARD average of 67%. This retrospective study reports on the outcomes of 1547 autologous ART treatments undertaken over a recent 3-year period. Based on various indications, cases were managed within 3 groupings - IVF Only, ICSI Only or IVF-ICSI Split insemination where oocytes were randomly allocated. Overall 567 pregnancies arose from mostly single embryo transfer procedures up to December 2016, with 402 live births, comprising 415 infants and a low fetal abnormality rate (1.9%) was recorded. When the data was adjusted for confounders such as maternal age, measures of ovarian reserve and sperm quality, it appeared that IVF-generated and ICSI-generated embryos had a similar chance of both pregnancy and live birth. In the IVF-ICSI Split model, significantly more ICSI-generated embryos were utilised (2.5 vs 1.8; $p < 0.003$) with productivity rates of 67.8% for pregnancy and 43.4% for livebirths per OPU for this group. We conclude that ART clinics should apply the insemination method which will maximize embryo numbers and the first treatment for unexplained infertility should be undertaken within the IVF-ICSI Split model. Whilst ICSI-generated pregnancies are reported to have a higher rate of fetal abnormalities, our data is consistent with the view that the finding is not due to the ICSI technique per se.

1. Introduction

The intra-cytoplasmic sperm injection (ICSI) technique was heralded as a distinct advance in the field of assisted reproductive technology (ART) as it enabled improved outcomes over the conventional in-vitro fertilization (IVF) procedure for those cases categorized as male-factor infertility [1]. However, concern was expressed as the selection technique for the individual spermatozoon to be injected, bypasses many natural biological processes that are thought to minimise the rate of embryonic and subsequent fetal anomalies. Such sperm-related natural conception processes include capacitation, hyper-activated motility, the acrosome reaction, cumulus dispersal, oocyte-induced sperm activation, zona-binding and sperm-egg membrane interaction causing cortical granule release [2]. However, the injection of a single spermatozoon immobilized by mechanical fracture of its tail, by-passes all of the "molecular passport" hurdles [3]. Hence the rational consideration prevailing to recent times was to avoid ICSI whenever it was deemed unnecessary. The pre-ICSI era data for fetal abnormalities showed higher rates for IVF infants, than those naturally conceived [4,5]. However, current data has seen this difference disappear, to be replaced by the ICSI-generated infants showing abnormality rates of 7.1% compared with 4.0% in the general population as well as for IVF-generated children [6]. Conversely, data from South Australia showed elevations for both IVF and ICSI against natural conceptions, but intriguingly this was only for younger women (< 30 years; OR 1.42); the difference disappearing for older women (35–39 years; OR 1.01) and actually reversing for women ≥40 years conceiving (most cases utilising ICSI; with OR 0.45; 95% CI 0.22–0.92) [7].

Despite the theoretical concerns, over the 25 years since its application, "current evidence suggests no difference in perinatal outcomes or congenital malformation risks in ICSI children when compared to naturally conceived children" [8]. Where elevated rates have been reported, such studies appear to have been affected by various confounders (including patient factors, ART confounders and study biases).

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Being of the view that ICSI is a safe procedure, our group has sought to extend its application outside male-factor and into the generally accepted non-male factor situations. These include paternal infections including HIV (to minimise the risk of disease transmission), preimplantation genetic diagnosis or screening (to exclude DNA contamination), as well as in donor programmes utilizing frozen spermatozoa or oocytes (to maximize fertilization opportunity in precious circumstances) [9,10].

To this date, a high specificity diagnostic test to predict optimal fertilization by IVF does not exist. A normal semen analysis profile according to WHO standards [11] has universally accepted limitations, and the widely adopted DNA fragmentation testing techniques have also fallen short on the anticipated expectations, despite providing some improvement in directing cases towards ICSI insemination [12]. Other techniques such as the hamster oocyte penetration test as well as a test of sperm binding to donor oocytes have met with significant ethical challenges in many countries, including Australia.

Having identified that DNA fragmentation testing also has limitations in predicting the likelihood of reduced fertilization of oocytes, even complete fertilization failure, we introduced the idea that all new cases entering ART programmes should be offered an ICSI-IVF Split fertilization "test", in order to diagnostically determine the optimum fertilization mode and to ensure the chance of fertilization for at least some eggs. Where IVF embryos were generated, this offered the opportunity for selecting such for embryo transfer (ET) if deemed of suitable quality. This proposal followed from documented cases of unexpected complete fertilization failure in couples with normal semen parameters and thus caused us to expand our indications for ICSI accordingly [13]. Ultimately, this has led to our clinic undertaking ICSI in 87% of patients where the overall rate for Australia and New Zealand currently stands at 67% [14].

This study reports on the outcomes of adopting a policy of offering all new couples with unexplained or poorly explained infertility, an IVF-ICSI Split protocol as a diagnostic exercise, thereafter applying IVF Only if that was clearly demonstrated to be optimal. The ICSI Only protocol applied for established indications, mainly male factor infertility but also included a range of other female considerations such as advanced age and low ovarian reserve. The outcomes of interest were to optimize the number of usable embryos which can be derived from a single oocyte pickup procedure (OPU) and the subsequent pregnancy and live births arising from those embryos; both fresh and frozen.

2. Materials and methods

2.1. Indications for ICSI

2.1.1. Male infertility factors

Most cases deemed to have a severe male factor were allocated to ICSI, although on occasion some severe male factor couples requested IVF or IVF-ICSI Split to determine if any IVF embryos could be created. The main male infertility factors for ICSI allocation included semen abnormalities, high rates of DNA fragmentation, males of advanced age and patients requiring surgical retrieval of sperm (7.4% of ICSI cases) including Vasal flush, MESA, PESA, TESA or micro-TESE (microsurgical epididymal sperm aspiration, percutaneous sperm aspiration, testicular sperm aspiration or microsurgical testicular sperm extraction, respectively).

The two sperm evaluation methods applied included semen analysis according to WHO criteria [11] and the DNA fragmentation index according to the Halo Test [15] or sperm chromatin structure assay; SCSA [16,17]. The primary criterion for deeming "significant male factor" related to the finding of diminished normal sperm morphology < 4% or a DNA fragmentation index of 15% (reduced from 30% following a research study at PIVET [13]). The fertilization chances with ICSI are improved where DNA fragmentation is shown, but high levels of fragmentation can compromise even the ICSI rates [17].

Other male medical conditions such as obesity [18,19], varicocele [20] and those with male reproductive tract issues such as past testicular trauma, antisperm antibodies, maldescent of testes, history of torsion, orchidopexy or even the isolated finding of reduced volume testes [21] were considered for ICSI. In addition, males with chronic disease especially involving chemotherapy or radiotherapy, or those with infectious disease such as HIV, Hepatitis B and Hepatitis C were also advised to use ICSI insemination. Finally, those on drug therapy which may affect fertilizing capacity such as sulphasalazine, cimetidine, allopurinol amongst others [22], along with males that use recreational drugs or are exposed to chemicals and heavy metals in "risky" occupations such as welding, were also advised ICSI.

2.1.2. Female factor

Advanced female age as well as reduced ovarian reserve are interconnected to lower antral follicle counts (AFCs) and low serum anti-Mullerian hormone (AMH). These combinations are closely associated with poor IVF prognosis, which includes an array of deficiencies such as reduced fertilization of oocytes and low oocyte numbers on retrieval. ICSI can at least improve oocyte fertilization, although it may not have any major influence over embryo quality or implantation potential. It does however reduce the problem of polyspermic IVF fertilization seen more frequently in oocytes from women of advanced age or diminished ovarian reserve [23,24].

It was reported by Diedrich's group that where < 4 oocytes are recovered, ICSI guarantees a successful treatment outcome more often than IVF and encourages the idea of milder forms of stimulation [25]. Our clinic has adopted a milder stimulation policy in recent years such that many women will now generate < 5 oocytes and ICSI provides a greater chance of generating embryos for transfer, especially if few oocytes [26] or only a single oocyte is retrieved [27].

Finally, oocyte anomalies are also linked to advanced female age and poor prognosis cases. Zona thickening is associated with advanced maternal age and zona hardening is associated with cryopreservation, especially for immature oocytes [28]. The consequential effect is reduced or failed fertilization [29] and this appears to be related to the degree of response to gonadotrophin stimulation. A number of zona problems can be encountered leading to reduced or absent sperm binding [30,31] and these can be resolved by ICSI [23].

2.1.3. Unexplained infertility

Whilst large RCT studies indicate that unexplained infertility is not, by itself an indication for ICSI, the outcomes of any IVF application may reveal a relevant "field trial" [32]. Reduced fertilisation rates < 50% of mature oocytes in either an IVF-all [33] or an IVF-ICSI Split "trial" indicates a need to apply ICSI for future IVF-related procedures [13]. The idea of applying an IVF-ICSI Split approach as a diagnostic exercise for all first-up cases of unexplained infertility has been demonstrated to be a cost-effective approach in the long term [34].

2.1.4. Intrauterine insemination failures

Cases who had failed to achieve a biochemical pregnancy following 2–6 cycles of intrauterine insemination (IUI) were advised to consider ICSI or at least IVF-ICSI Split from our internal studies [35] and indicated by others [36,37]. From internal studies the fertilization rate of cases proceeding to IVF from failed IUI was significantly lower than those directly utilizing ICSI (49% vs 69%; $p < 0.001$), and occurrences of complete fertilisation failure were significantly higher (13.4% vs 2.9%; $p < 0.001$) causing a change in policy to recommend ICSI in such cases.

2.1.5. Genetic analysis of embryos

Where preimplantation diagnosis (PGD) and screening (PGS) was applied, the current recommendations are to utilize ICSI in order to avoid contamination of the embryo biopsy specimens (either blastomeres or trophoblast specimens) from sperm adherent to the zona