Symposium

In vitro fertilization/intracytoplasmic sperm injection for male infertility

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ABSTRACT

Progress in the field of assisted reproduction, and particularly micromanipulation, now heralds a new era in the management of severe male factor infertility, not amenable to medical or surgical correction. By overcoming natural barriers to conception, in vitro fertilization and embryo transfer (IVF-ET), subzonal sperm insemination, partial zona dissection, and intracytoplasmatic injection of sperm (ICSI) now offer couples considered irreversibly infertile, the option of parenting a genetically related child. However, unlike IVF, which necessitates an optimal sperm number and function to successfully complete the sequence of events leading to fertilization, micromanipulation techniques, such as ICSI, involving the direct injection of a spermatozoon into the oocyte, obviate all these requirements and may be used to alleviate severe male factor infertility due to the lack of sperm in the ejaculate due to severely impaired spermatogenesis (non-obstructive azoospermia) or non-reconstructable reproductive tract obstruction (obstructive azoospermia). ICSI may be performed with fresh or cryopreserved ejaculate sperm where available, microsurgically extracted epididymal or testicular sperm with satisfactory fertilization, clinical pregnancy, and ongoing pregnancy rates. However, despite a lack of consensus regarding the genetic implications of ICSI or the application and efficacy of preimplantation genetic diagnosis prior to assisted reproductive technology (ART), the widespread use of ICSI, increasing evidence of the involvement of genetic factors in male infertility and the potential risk of transmission of genetic disorders to the offspring, generate major concerns with regard to the safety of the technique, necessitating a thorough genetic evaluation of the couple, classification of infertility and adequate counseling of the implications and associated risks prior to embarking on the procedure. The objective of this review is to highlight the indications, advantages, limitations, outcomes, implications and safety of using IVF/ICSI for male factor infertility to enable a more judicious use of these techniques and maximize their potential benefits while minimizing foreseen complications.

Key words: Assisted reproductive technology, azoospermia, *in vitro* fertilization, intracytoplasmic sperm injection, male factor infertility, microsurgical sperm extraction, oligoasthenoteratozoospermia, preimplantation genetic diagnosis, sperm cryopreservation

INTRODUCTION

The role of assisted reproduction for male infertility

Until recently, the primary treatment option for infertile men with obstructive azoospermia was vasovasostomy or vasoepididymostomy for reconstructable causes, or implantation of an alloplastic artificial spermatocele for subsequent percutaneous retrieval of sperm for unreconstructable causes, such as congenital absence of the vas deferens. [1] Since the first US report of a successful delivery from *in vitro* fertilization (IVF) in 1982, progress in the field of assisted reproduction and micromanipulation has been truly dramatic, particularly in the area of male factor infertility, offering couples considered irreversibly infertile and eligible for donor insemination or adoption, the option of parenting a genetically related child despite severe impairments in sperm quantity and quality.[2] Assisted reproductive techniques that aim to overcome natural barriers to fertilization include intra-uterine insemination (IUI), *in vitro* fertilization and embryo transfer (IVF-ET), gamete intrafallopian transfer (GIFT), subzonal sperm insemination (SUZI), partial zona dissection (PZD), and intracytoplasmic sperm injection (ICSI).[3] Though technology has evolved, IVF failed to solve problems concerning sperm and IVF-ET as treatment for male

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factor infertility is associated with low fertilization and pregnancy rates than for other indications.[4] Though SUZI and PZD facilitated sperm access to the oocyte, they resulted in only a marginal improvement in conventional IVF results as relatively large numbers of sperm were still required and cases with a very limited number of spermatozoa in the ejaculate could still not be treated, fertilization rates remained low, while rates of polyspermic fertilization increased.[4]

The advent of ICSI in 1992, involving the injection of a single sperm (or sperm head or nucleus) into the oocyte is an important breakthrough that has revolutionized the treatment of male infertility and resulted in the widespread use of this technique world over[5] [Figure 1]. Analysis of data from National and Regional registers for trends in the use of ICSI and indications for assisted reproductive technology (ART) show that the use of ICSI has increased from 39.6% of ART cycles in 1997 to 58.9% in 2004[6] relegating varicocele repair, vasectomy reversal, diagnosis and treatment of ejaculatory duct obstruction. ICSI can be carried out with fresh and frozen-thawed epididymal sperm following microepididymal sperm aspiration (MESA) or testicular sperm following percutaneous sperm aspiration (PESA), testicular sperm extraction (TESE), and modified percutaneous sperm aspiration in patients with obstructive azoospermia (OA), and with testicular sperm in some patients with non-obstructive azoospermia (NOA)[7] with pregnancy rates up to 52% and ongoing pregnancy and live delivery rates as high as 37% per cycle attempt.[2]

However, not all men having impaired semen parameters are ideal candidates for ICSI for numerous reasons including a lack of addressing the underlying problem causing the male infertility, unknown genetic consequences, and cost-effectiveness issues.[8] Technical, biological and genetic hazards associated with ICSI are causes for concern. [9]

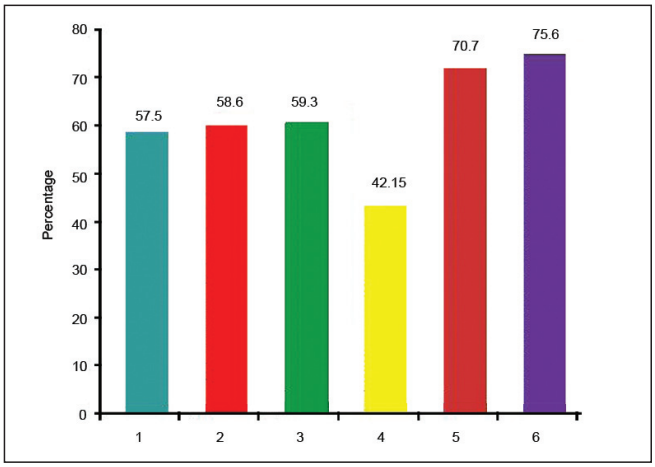

Figure 1: Analysis of data from National and Regional registers for trends in the use of ICSI. 1. USA, 2. Australia/New Zealand, 3. Europe, 4. The Nordic countries, the Netherlands and the UK (40.0-44.3%), 5. Austria, Belgium and Germany (68.5-72.9%), 6. Southern European - Greece, Italy and Spain (66.0-81.2%)

CLINICAL DISCUSSION

Evaluation of the male partner

The first step in evaluation is a thorough history and physical examination with initiation of basic laboratory studies.[21] Evaluation of sperm function, involving the ability to achieve sperm-ZP binding, undergo the acrosome reaction, penetrate the ZP and fuse with the oolemma is essential to assess sperm fertilizing ability in standard IVF. However, this is not so in ICSI since sperm bypass the ZP and oolemma during the injection of a single spermatozoon directly into oocyte cytoplasm.[22] The combination of semen analysis with advanced sperm function tests (sperm-ZP binding, sperm-ZP penetration, ZP-induced AR tests) provides important diagnostic and prognostic information for male infertility, not obtained in conventional semen analyses alone and is crucial in the decision-making process regarding the ART technique (IVF or ICSI) to be employed.[22] Additionally, an evaluation of chromatin structure/sperm DNA damage, reported as a probable cause of 20% of male infertility and a factor influencing accurate transmission of paternal genetic information and sperm fertilizing ability[23] appears to be a useful tool for assessing male fertility potential both *in vitro* and *in vivo*.[24,25] Moreover, genetic karyotyping and screening of patients with severe male infertility (OATS, cryptozoospermia, non-obstructive azoospermia) for chromosome abnormalities, Y chromosome microdeletions involving the AZFc region, cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations, and androgen receptor gene mutations prior to conducting ICSI has prognostic implications. While preventing the unintended vertical transmission of these disorders to the offspring, these tests would aid in pre-procedure counseling.

Additionally, in order to maximize the treatment outcome following ICSI, a thorough genetic evaluation of the female partner, particularly in women with advanced age, must be carried out.

The infertility practitioner should have a thorough understanding of the advantages and limitations of various laboratory tests as well as the indications, costs and success rates of all treatment options.[21]

SPERM PREPARATION FOR ART

The tilt of ART indications from mere gynecological towards predominantly andrological indications necessitated the development of more sophisticated techniques to separate functional spermatozoa from those that are immotile, have poor morphology or that are not capable of fertilizing oocytes.

Sperm preparation techniques, such as the conventional swim-up procedure, migration-sedimentation, glass wool filtration or density gradient centrifugation, that may generally be used for IVF for mild to moderate male factor infertility depending on the total motile sperm count in the ejaculate at an initial evaluation, may rarely be applicable to severe male factor infertility (cryptozoospermia, OAT, oligoasthenozoospermia, obstructive and non-obstructive azoospermia) that would necessitate microsurgical sperm retrieval techniques to obtain adequate sperm for ART.[26]

Microsurgical sperm extraction

Depending on the type of azoospermia (obstructive/non-obstructive), sperm can be retrieved for ICSI from either the epididymis by MESA, the testis by aspiration from the testicular parenchyma (TESA), or by a surgical biopsy (TESE) using the conventional or microdissection technique. The indications for MESA and TESE are presented in Table 1. In combination with ART procedures, like IVF and ICSI, and depending on the indication, these microsurgical procedures have enlarged the therapeutic options for irreparable azoospermia,[10] the most severe form of male factor infertility, present in approximately 5% of all investigated infertile couples.[27]

Since epididymal sperm may not always be available, necessitating testicular sperm retrieval,[28] epididymal sperm retrieval should be performed only when micromanipulation is available in conjunction with IVF to maximize the chances of fertilization and subsequent pregnancies.[29] The use of epididymal sperm in conjunction with ICSI maximizes the chances of pregnancy in couples with obstructive azoospermia with better fertilization rates per oocyte (45%), and clinical (52%) and delivery rates per cycle (48%) following IVF+ICSI than IVF alone.[30]

Testicular sperm retrieval

The successful application of TESE depends on the identification of seminiferous tubules with focal spermatogenesis in patients with NOA (Sertoli-cell only, or maturation arrest, due to the absence of spermatogenesis or to a block in meiosis)[31,32] as the testicular tubules of patients with NOA are usually heterogeneous, and TESE may not always be successful in these patients.[32]

Successful sperm retrieval following TESE has been reported in 30% of the patients with Sertoli-cell-only syndrome,[33] 86% patients with spinal cord injury (SCI) and 50% of patients with a history of orchidopexy, the sperm recovery rate and the ICSI cycle outcome being similar to the population of men with non-obstructive azoospermia.[13] Conventional TESE, when combined with MD-TESE, results in an improvement in sperm retrieval rates from 24% to 48% in NOA patients with a 90% clinical pregnancy rate (CPR) following TESE-ICSI.[34] Pregnancy rates as high as 45% have been reported following microdissection TESE-ICSI in 60% of men with NOA, including 70% of difficult cases, such as men with Klinefelter's syndrome.[35] Microdeletion of the entire AZFa or AZFb regions of the Y chromosome portends an exceptionally poor prognosis for sperm retrieval, whereas the majority of men with AZFc deletions have sperm within the semen or testes available for use in IVF/ICSI.[36] However, they will transmit the deletions to their male offspring by intracytoplasmic sperm injection.[37]

Table 1: Indications for microfertilization-intracytoplasmic sperm injection

Obstructive azoospermia[10] (following MESA/TESE) in patients with
- Obstructive azoospermia[10] (following MESA/TESE) in patients with
- Congenital absence of the vas deferens (CAVD)
- Acquired vas obstruction
- Irreparable epididymal obstruction
- Postinfectious epididymal obstruction
- Conservatively untreatable ejaculatory disturbances
- Failed microsurgical reversal for vasectomy

Non-obstructive azoospermia (following TESE) in patients with
- Germ-cell aplasia, maturation arrest, and tubular sclerosis/atrophy, all with
- Focal spermatogenesis[11]
- Sertoli-cell only syndrome[12]
- Persistent azoospermia post chemotherapy[12]
- A history of orchidopexy[13]
- Spinal cord injury[14]
- Seminiferous tubule dysgenesis (Klinefelter syndrome 47, XXY)[15]

Presence of acrosomeless or immotile spermatozoa[16]

High risk of fertilization failure due to
- Subnormal sperm samples -semen parameters below the threshold for standard IVF treatment e.g. oligoasthenoteratozoospermia (OAT)
- Severely oligozoospermic and teratozoospermic men (strict normal sperm morphology ≤5%) with a very high (>70%) frequency of defective sperm-zona pellucida (ZP) interaction and hence a high risk of low or zero fertilization rate in IVF.[17,18]
- Sperm autoimmunity (high titers of antisperm antibodies/sperm-bound antibodies- interference with gamete interaction)[16,19]

Two previous fertilization failures with conventional IVF[16]

When preimplantation genetic diagnosis (PGD) is indicated in pregnancies that are at high risk of aneuploidy because of genetic factors associated with azoospermia, to avoid contamination by extraneous DNA in the case of Polymerase chain reaction (PCR)- based testing and to increase the number of embryos available for testing.[20]

In vitro maturation of testicular retrieved sperm results in a remarkable increase in sperm motility after 24 h of retrieval, with a maximum motility rate between 48 and 72 h of culture, motile spermatozoa being observed up to 120 h in culture.[38]

Randomized controlled trials (RCTs), comparing the effectiveness of different sperm retrieval techniques in men with azoospermia, suggest that there is insufficient evidence to recommend any specific sperm retrieval technique for azoospermic men undergoing ICSI, the least invasive and simplest technique available being recommended, and the classification of azoospermia (OA/NOA) and its cause being most relevant to a successful clinical outcome.[27]

IVF TECHNIQUE

Oocytes are retrieved by transvaginal oocyte recovery following hormonal stimulation of the female patient with an optimum stimulation protocol. Following sperm preparation, the oocytes in microdroplets of culture medium in a culture dish, are inseminated with an adequate number of sperm and incubated overnight in the CO2 incubator under ideal temperature and pH conditions. Fertilization is assessed under a stereozoom microscope on the next day by the presence of two pronuclei and two polar bodies [Figure 2] 16-20 h post insemination. The fertilized oocytes are transferred into microdroplets of fresh embryo culture medium and incubated for another 24 h. Following a morphological evaluation of embryo cleavage, four-cell Grade A embryos [Figure 3] are selected, drawn in an embryo transfer catheter, and transferred atraumatically into the patient under sonographic guidance. Alternatively, embryos may be further cultured to the eight-cell stage [Figure 4] or to the blastocyst stage [Figure 5] and transferred on Day 3 or Day 5, respectively. The optimum number of embryos to be transferred is decided by the clinician and is bound by the regulations in force, but normally does not exceed three. A pregnancy may be confirmed by beta human chorionic gonadotropin (b-hCG) evaluation two weeks after IVF or the presence of a gestational sac on ultrasonography four weeks after the procedure.

Intracytoplasmic sperm injection technique [Figures 6-12]

Except for the instrumentation and specific fertilization technique, the steps involved in ICSI are similar to those in IVF. The micromanipulation technique involves the use of an ICSI micromanipulation microscope with a holding pipette to hold the oocyte during injection and an injection pipette to inject the spermatozoon into the ooplasm. A single ejaculated, epididymal or testicular viable spermatozoon is inactivated in sperm immobilizing medium, washed in culture medium and drawn into the injection pipette with a little medium. Holding the oocyte in medium with holding pipette, the injection pipette is pierced through the oolemma, a little ooplasm drawn into the injection pipette and the spermatozoon along with the ooplasm injected into the oocyte. The remaining steps follow as in IVF. Figure 13 depicts an eight-cell Grade A ICSI embryo. A pregnancy may be confirmed by a beta hCG evaluation two weeks after ICSI or the presence of a gestational sac on ultrasonography at least four weeks after procedure.

Factors affecting the outcome of ART procedures

The most significant factors that influence the outcome of ART include i) the technique used, ii) technical factors, iii) the indication for ART, iv) sperm motility and maturity, v) pretreatment with medical or surgical therapy.

Technique

While fertilization rates with MESA-IVF are low despite large numbers of epididymal sperm at retrieval in patients with obstructive azoospermia due to congenital bilateral absence of the vas deferens (CBAVD), no fertilization has ever been possible with TESE-IVF. IVF with ICSI yields good clinical results in couples with severe male factor infertility.[1,39] Because of the consistently good results with MESA/TESE-ICSI when compared with conventional IVF, ICSI is mandatory for all future MESA patients.[31]

Technical factors

Technical factors critical for achieving high rates of fertilization and pregnancy include the use of standardized ICSI pipettes, the immobilization of the spermatozoon before injection, and the aspiration of a minimal amount of ooplasm before reinjection with the sperm.[40]

Indication

Male vs. non-male factor

Couples with previous failed fertilization or a low fertilization rate without a male factor have significantly lower pregnancy and implantation rates compared to couples with a male factor despite similar fertilization and cleavage rates and a similar number and morphological grade of embryos transferred in both the groups.[41, 42] The significantly smaller chance of conceiving after subsequent ICSI probably reflects intrinsic oocyte defects not bypassed by ICSI.[42]

Male factor

There are conflicting reports with regard to the TESE-ICSI outcome in patients with OA and NOA. While some studies have reported no differences in fertilization and pregnancy rates following the use of fresh[42,43] or cryopreserved[44] motile testicular sperm, regardless of the underlying pathology, source, or the quantity of sperm,[44] others have reported significantly lower fertilization (48.5% vs. 59.7%) implantation (8.6% vs. 12.5%) and clinical pregnancy (15.4% vs. 24.0%) rates per cycle following TESE-ICSI in men with NOA compared to those with OA.[11] Significantly lower pregnancy rates have been reported with frozen-thawed

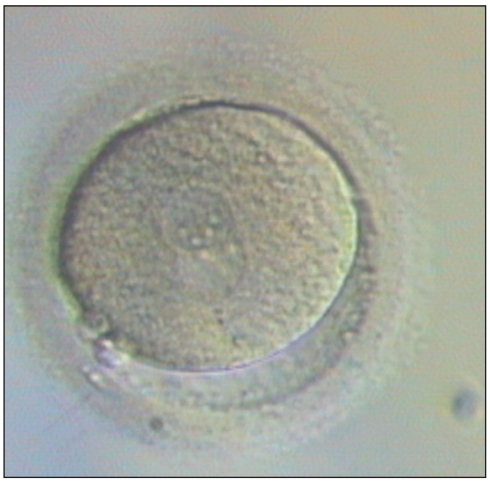

Figure 2: Fertilized oocyte with 2 pronuclei and 2 polar bodies

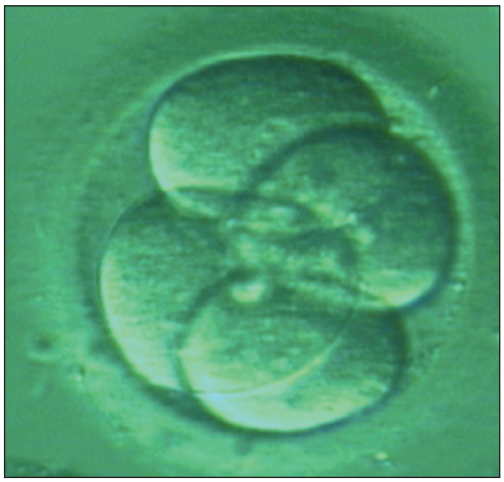

Figure 3: Day 2, 4-cell embryo

Figure 4: Day 3, 8-cell embryo

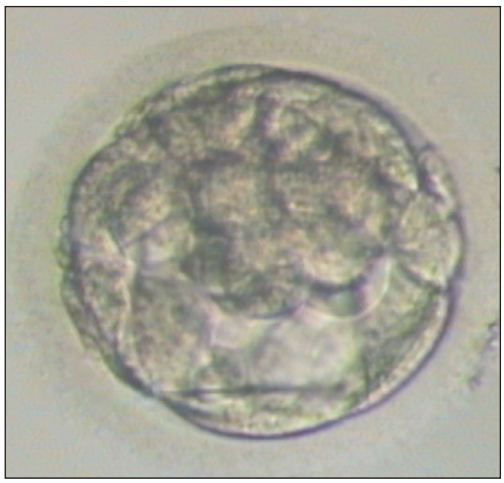

Figure 5: Day 5, Blastocyst

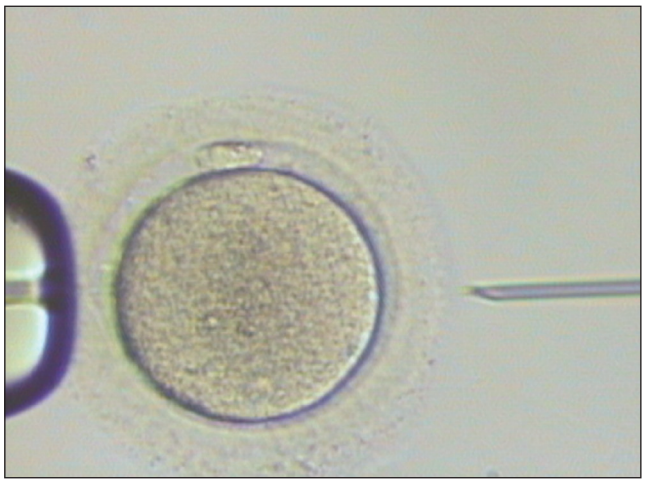

Figure 6: Mature oocyte ready for ICSI with the holding pipette (left) and injection pipette (right) in place

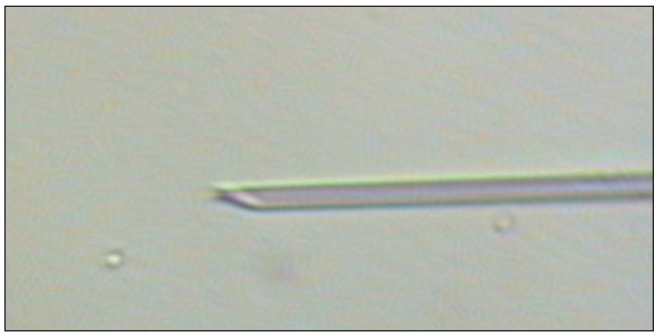

Figure 7: Sperm aspiration into the injection pipette

testicular sperm from patients with NOA compared to OA (9.1% vs. 46.2%),[45] and often, complete fertilization failure and limited overall success rate observed, with ongoing pregnancies in ≤ 20% of ICSI cycles despite the high sperm retrieval rates following TESE.[14] The only significant factors affecting the outcome were maternal age, the number of embryos transferred and the application of assisted hatching.[45,46] However, pregnancy rates per testicular sperm- as high as 64% (fresh sperm) and 25% (frozen-thawed) have been reported in patients with SCI following TESE-ICSI.[14] Though pregnancy and birth may be

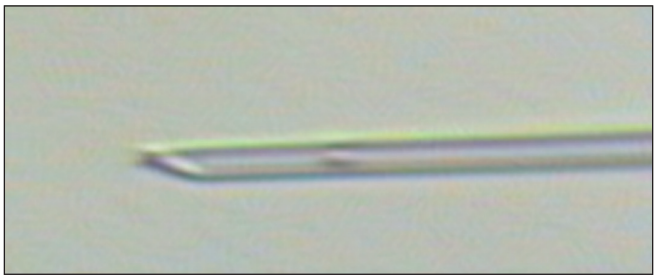

Figure 8: Sperm aspiration into the injection pipette

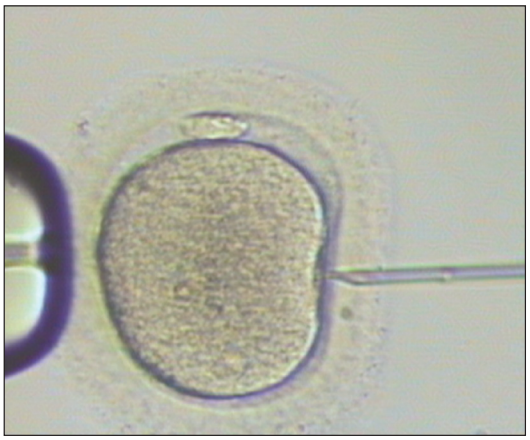

Figure 9: Injection of the spermatozoon into the oocyte

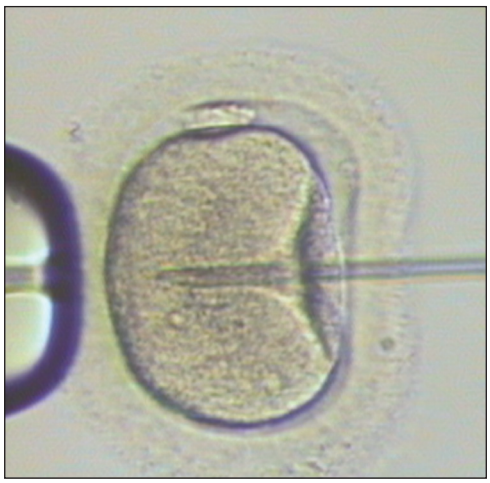

Figure 10: Injection of the spermatozoon into the oocyte

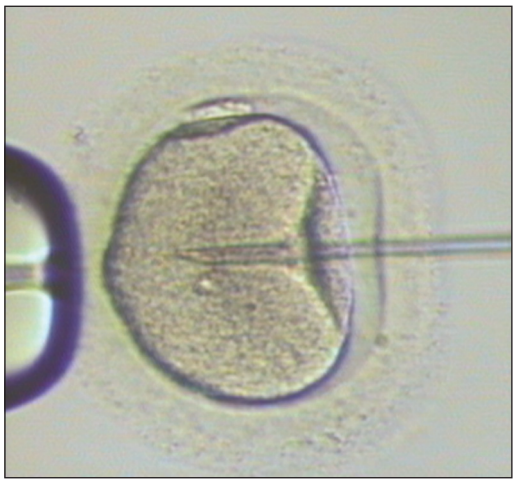

Figure 11: Injection of the spermatozoon into the oocyte

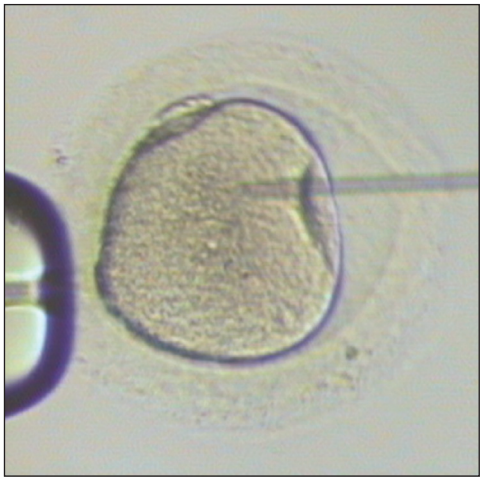

Figure 12: Withdrawal of the injection pipette

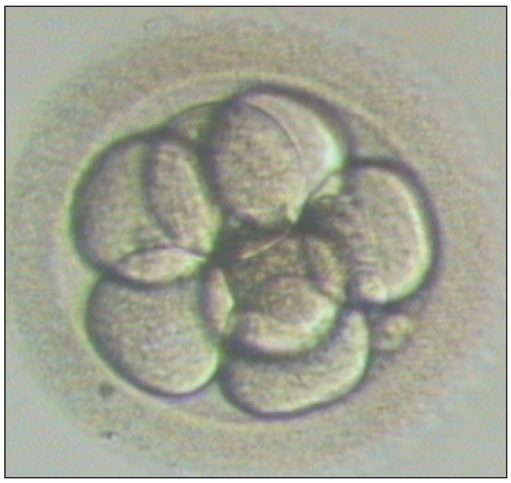

Figure 13: Day 3, 8-cell Grade A ICSI embryo

attained in azoospermic non-mosaic Klinefelter's individuals following TESE-ICSI,[46] the birth rate is very low compared with the fertilization rate, suggesting an increased risk of chromosomal abnormalities.[47]

Sperm parameters

The outcome of ICSI is influenced by sperm motility of microsurgically retrieved fresh as well as frozen-thawed sperm[23,48] and the maturity of sperm selected.[49] Significantly higher fertilization (77.0% vs. 29.3%) and pregnancy rates (44.3% vs. 20.0%) have been reported following the use of fresh motile vs. nonmotile sperm and cryopreserved motile vs. non-motile sperm (fertilization rates: 70.0% vs. 50.9%; pregnancy rates 33.9% vs. 27.3%) following TESE-ICSI[22] and MESA-ICSI (fertilization rates: 68.4% vs. 31.6%, respectively;

$P < 0.01$)[48] suggesting that motile sperm are necessary for optimal fertilization and pregnancy outcomes.[23]

Pretreatment with medical therapy

Preoperative evaluation of consequent treatment of antibody-positive men with low-dose intermittent prednisone prior to MESA-IVF results in a significant improvement in fertilization (39% vs. 21%, $P < 0.0001$) and pregnancy (48% vs. 26%, $P = 0.06$) rates compared to no treatment. Empiric treatment with prednisone may be detrimental to the fertility of men who have no antisperm antibodies. [50] Higher sperm retrieval rates (77% vs. 55%) have been reported in men with hypogonadism, who respond to medical therapy (aromatase inhibitors, clomiphene citrate or human chorionic gonadotropin) with a resultant testosterone ≥ 250 ng/dL compared to men in whom post-treatment testosterone was < 250 ng/dL. Men with normal baseline testosterone had the best sperm retrieval rate of 86%.[51]

Pretreatment with surgical therapy

Significantly higher sperm retrieval rates (53% vs. 30%; $P = 0.036$) and clinical pregnancy rates (31.4% and 22.2%; $P>0.05$) have been reported following microsurgical TESE in patients with clinical varicocele and NOA who underwent varicocelectomy despite similar fertilization and embryo transfer rates following TESE-ICSI.[52] However, the degree to which varicocele repair improves pregnancy rates and the success of assisted reproductive technology remains controversial.[53]

Neither the source of sperm (epididymal/ testicular), the cause of obstruction [congenital absence of the vas deferens (CAVD) or failed vasoepididymostomy)][28,31] or the state of sperm (fresh or frozen-thawed epididymal[29,54-56] or testicular[23,38,57-61]) affect the pregnancy outcome following ICSI, despite an impairment in sperm motility following cryopreservation and more favorable implantation rates in the fresh testicular sperm group,[38,60] the only significant factor being the age of the female.[31] High normal fertilization, cleavage, and PRs have been reported following MESA-ICSI or TESE-ICSI with fresh or frozen-thawed epididymal and testicular spermatozoa.[62] While studies have reported no difference in fertilization rates following MESA/TESE-ICSI,[63] significantly lower fertilization rates have been reported following TESE-ICSI or MESA-ICSI compared to ICSI with ejaculated sperm.[62-64] While some studies have also reported a significantly lower number of embryos transferred,[62,63] others have reported no differences in the embryo quality, the percentages of transfer after ICSI or the clinical pregnancy rates following ICSI with ejaculated, epididymal or testicular sperm.[64]

THE VALUE OF SPERM CRYOPRESERVATION

Epididymal sperm cryopreservation in patients with obstructive azoospermia[56] and freezing and *in vitro* culture of testicular biopsy tissue in patients with NOA,[38] using a simple freezing protocol, is a feasible and efficient option that offers several advantages such as avoidance of repeated scrotal surgery,[56] pointless ovarian stimulation in the female partner,[65]and the opportunity of performing several IVF/ICSI treatments from a single sample at later dates or in other centers without jeopardizing the ICSI success rate,[38,54] while optimizing the pregnancy outcome.[29]

In vitro fertilization vs. intracytoplasmic injection of sperm

A systematic review of eight randomized studies comparing ICSI with conventional IVF reported evidence of significantly better fertilization rates with ICSI than IVF in couples with borderline semen (concentration 10-20 mill/mL, motility 30-50%, morphology 4-14% normal forms) but no evidence of a difference in fertilization rates per retrieved oocyte or pregnancy rates between ICSI and conventional IVF for couples with normal semen (concentration >20 mill/mL, motility >50%, morphology >14%).[4] No difference between the IVF and ICSI outcomes have been reported in men with oligozoospermia but ICSI showed a significant advantage over IVF in patients with asthenoteratozoospermia and obstructive azoospermia, and was the only option in non-obstructive azoospermia in achieving an acceptable pregnancy rate.[66] Intracytoplasmic sperm injection resulted in fertilization rates of 50-60%, overall pregnancy rate of 24.9% per embryo transfer, and live pregnancy rate per started cycle of 13.4% in cases where IVF had failed despite normal sperm quality.[67]

With regard to the risk of nuclear spindle damage following ICSI, the incidence of non-disjunction in oocytes fertilized by conventional IVF was significantly lower (20.0%, $P < 0.01$), suggesting that ICSI might interfere with regular chromosome segregation at the second meiotic division of the oocytes.[68] However, the majority of studies on ICSI and IVF offspring have, setting aside inconsistencies in methodology and classification, not shown significant differences between the two techniques in terms of congenital abnormalities (between 3 and 4%), despite a slightly increased risk of *de novo* chromosomal abnormalities compared to naturally conceived offspring.[9,69] The risk for congenital malformations following IVF was reported to be well within the basic background risk for congenital malformations.[70] No differences in behavioral and psychological development have been reported in children conceived following IVF/ICSI compared to naturally conceived children.[8]

Repeat cycles

A lower rate of ongoing pregnancies per patient has been reported following IVF (24.9%) compared to after ICSI (32.9%), however, it was similar or even slightly increased in patients with more than one attempt. On the other hand, there was a high pregnancy rate with ICSI in the first two cycles (35.9%), but patients with more than two ICSI cycles

had a significantly lower chance of conceiving (20.7%) in the absence of confounding factors, suggesting a possible negative selection of patients with poor embryo quality and previously failed attempts after ICSI, possibly due to an andrological factor involving chromosomal or genetic disturbances in spermatozoa that could be the reason for failure.[71]

COMPLICATIONS

Following IVF/ICSI with ejaculate, epididymal, and testicular sperm, complications, such as ectopic pregnancy (1.9%), heterotopic pregnancy (0.2%), abortion (20.6%), multiple pregnancy (28%), pregnancy-induced hypertension (10%), preterm labor (21.5%), low birth weight (30.5%), and intrauterine death (9.95%) have been reported necessitating well-defined indications, proper patient monitoring and precautionary measures.[72]

LONG-TERM OUTCOME FOLLOWING INTRACYTOPLASMIC INJECTION OF SPERM

More than a decade after its introduction, the possible adverse effects of ICSI are still debated and ICSI continues to raise concerns because of the mechanical perforation of the oocyte, the possible transmission of foreign genetic material, the use of immature or senescent germ cells, the association between genetic disorders and some forms of male infertility,[73] increase in childhood illness observed and the future fertility of these children.[74] ICSI outcome studies indicate a significant increase in prematurity, low birth weight, and perinatal mortality associated with single and multiple births, similar to the outcomes of conventional IVF[20] and poor perinatal outcomes compared to natural conception.[75]

Some follow-up studies of children born after ICSI report no significant differences in the incidence of major congenital malformations, or major health problems in the first year of life,[76] up to age 5-8 years,[75] in the occurrence of vision or hearing impairments at a mean age of 5.5 years,[77] or in the developmental outcomes (cognitive and motor development) of 10-year-old singletons[78] when compared with children conceived by routine IVF or spontaneous conception. Findings in 10-year-old singletons were in line with those obtained at age 8.[78] No discrepancies in the neurodevelopmental outcome of these children have been established and no detrimental psychological effects on the families have been reported.[74] However, others have reported an increased risk of imprinting disorders,[79] congenital malformations and chromosome aberrations[80-82] in children born following ICSI. The increased risk of perinatal morbidity, mortality, and congenital malformations associated with singleton births has been linked to the infertility of the couple and the background risks rather than the techniques used.[73, 74] Whether ICSI will eventually perpetuate male infertility is far from clear, because at present the inheritance pattern of idiopathic male infertility is unknown.[73]

In the light of the available evidence, ICSI is considered a safe procedure provided this treatment is performed in clinics with the highest standards of expertise and with a continuous follow-up program for the offspring. As long as follow-up studies have a limited power to detect small increases in malformations and as long as no information is available on long-term and next-generation cohorts, ICSI must be used with caution only when no alternative evidence-based therapy is available [73] and only after performing PGD in cases with a high risk of transmission of genetic disorders. Further follow-up of these children is needed to fully establish the long-term health implications of IVF and ICSI.[74]

THE SIGNIFICANCE OF PREIMPLANTATION GENETIC DIAGNOSIS

The dramatic increase in the worldwide use of ART (1–3% of births),[79] increasing evidence of several genetic abnormalities [karyotype abnormalities, Y chromosome microdeletions involving the AZFc region, cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations, androgen receptor gene mutations] in infertile men with numerical or structural sperm defects,[83] vertical transmission of Yq deletions,[84] increased risk of imprinting disorders (Beckwith-Wiedemann syndrome and Angelman syndrome, Silver-Russell syndrome, maternal hypomethylation syndrome, and retinoblastoma) [79] and congenital malformations and chromosome aberrations[80-82] in children born following ICSI, highlight the hazards associated with ICSI despite its documented efficacy and success in the treatment of severe male infertility. Concerns have been raised about the possibility that sperm with DNA fragmentation may be involved in the fertilization process during ICSI.[83] Chromosome aberrations, Y chromosome microdeletions and CFTR mutations alone may explain up to 25% of azoospermia and severe oligozoospermia.[85]

Preimplantation genetic diagnosis involves the detection of affected embryos before implantation by fluorescent *in situ* hybridization (FISH) for X-linked diseases and numerical and structural chromosomal disorders, or by polymerase chain reaction (PCR) for monogenic disorders (including some X-linked diseases).[86] potential health hazards associated with ICSI. Though PGD has been indicated as a feasible technique by which to avoid the birth of genetically affected children to couples at risk following ICSI and is increasingly being used, though its effectiveness is far from clear[87] definitive conclusions are difficult to substantiate due to the rarity of imprinting disorders and the variability in ART protocols.[79] To date, the vast majority of children conceived

using these ART techniques are apparently normal.[88] However, couples undergoing ART for male factor infertility should be counseled about the risks of transmission of these disorders to the offspring, the possibility of genetic testing, and the implications of the results for the patient.[85]

RECENT ADVANCES

Laser-assisted ICSI (through a laser-drilled hole in the zona pellucida) has been proposed as a suitable alternative to conventional ICSI for reducing technique-related oocyte damage in oocytes with a propensity for sudden oolemma breakage during conventional ICSI or where only few oocytes are available, with better oocyte survival rates (97.9% versus 85.7%; $P<0.05$), tendency to form more two-pronucleated zygotes (78.6% versus 69.2%; $P=0.07$) and less zygotes with three or more pronuclei (2.8% versus 7.8%; $P=0.06$) as compared with sibling oocytes treated by conventional ICSI (n=140).[89]

Artificial oocyte activation with calcium ionophore A23187 has been suggested as a means of coping with the serious psychological consequences in the event of fertilization failure following ICSI and may improve ICSI outcomes in patients with azoospermia following MESA,[90] teratozoospermia,[91] and dysfunctional sperm, characterized by ultrastructural and protein expression anomalies and a low fertilization rate in ICSI.[92] The fertilization rate of oocytes activated with calcium ionophore (80.0%) was higher than that of the non-activated oocytes (25.0%).[92]

'Genetic sonography', i.e. high-resolution ultrasound with measurement of nuchal translucency at the end of the first trimester, and detailed fetal evaluation at 18-22 weeks of gestation may be used as an alternative to invasive prenatal diagnosis for chromosome abnormalities to overcome the potentially associated ethical problems.[93]

CONCLUSION

By addressing the gamete per se i.e. the spermatozoon, rather than the medium that carries it, ICSI has superseded IVF in its extent of application and emerged as a promising technique to alleviate severe male infertility due to a fleet of causes, including those of genetic origin following microsurgical sperm retrieval by MESA/TESE, offering couples the opportunity to parent a genetically-related child. The sperm retrieval technique employed depends entirely on the indication and there is insufficient evidence to recommend any specific sperm retrieval technique for azoospermic men undergoing ICSI, the least invasive and simplest technique available being recommended. However, TESE appears to be more beneficial in cases of non-obstructive azoospermia. The most significant factors that influence the outcome of ART include i) the technique used, ii) technical factors, iii) the indication for ART and appropriate patient selection, iv) sperm motility and maturity, and v) pretreatment with medical or surgical therapy. Neither the source of sperm (epididymal/testicular), the state of sperm (fresh or cryopreserved), nor the cause of obstruction influence the ART outcome, the age of the female being the only significant factor. However, outcomes may be inferior to those obtained with ejaculate sperm. ICSI alleviates severe male factor infertility following failed IVF attempts but repeat cycles may yield a poor prognosis compared to IVF. No differences in terms of congenital abnormalities, behavioral and psychological development have been reported in children conceived following IVF/ICSI compared to naturally conceived children. Epididymal sperm cryopreservation, freezing and *in vitro* culture of testicular biopsy tissue have logistic advantages in azoospermic patients in maximizing outcomes and facilitating future treatments.

However, increasing evidence of a genetic involvement in severe male infertility and the biological plausibility of transmitting genetic disorders to the offspring, mandate a comprehensive male infertility evaluation including a physical examination, history-taking, semen analyses, sperm function tests, and genetic testing where indicated despite a lack of consensus on the application and efficacy of PGD. Financial, psychological, procedure and implication counseling should compulsorily be offered to couples undergoing ART for severe male factor infertility. Indications for ICSI should be better defined and randomized controlled trials conducted to fully evaluate the implications of ICSI and the value of genetic screening in order to enable a more judicious use of the technique to maximize the benefits while minimizing the potential complications. There is a need for an improved understanding of the mechanisms of imprinting at the molecular level so that methods to prevent disruption of this critical epigenetic process can be developed.[94]

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