RECENT ADVANCES IN MALE FACTOR INFERTILITY AND INTRACYTOPLASMIC SPERM INJECTION

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The first trial of recovering a preimplantation stage embryo from flushing of a rabbit oviduct and transferring the embryo to a foster mother was demonstrated by Walter Heape in 1890 (1). However, Chang et al (2) in 1959 reported the first successful fertilization of rabbit eggs in vitro. The first successful human pregnancy and birth after in vitro fertilization (IVF) was reported by Steptoe and Edwards in 1978 (3). IVF by insemination has been used to treat couples with male factor infertility for over a decade with relatively disappointing results, presumably because the sperm cells were unable to penetrate the zona pellucida and fuse with the oolemma. This has led to the application of gamete micromanipulation. The initial approach to assisted fertilization was to partially bypass the zona pellucida. An animal model developed by Gordon and Talansky (4) demonstrated that an artificial opening introduced in the zona pellucida before insemination enhanced fertilization. Partial zona dissection (PZD) involved mechanical opening of the zona pellucida by a narrow microneedle while the oocyte was suspended in a hyperosmatic solution. Unfortunately this technique has not been successful. Fertilization rates were very low and there was a relatively high incidence of polyspermy (the penetration of more than one spermatozoan).

To overcome these limitations it appeared necessary to bypass the zone pellucida, so the next method entailed microsurgical placement of sperm in the perivitelline space of the oocyte, subzonal insemination (SUZI). Ng et al (5) were the first to report its successful clinical application. The greatest weakness of such zona bypass methods was their inefficiency, as they failed to achieve normal fertilization rates beyond 20% and produced high rates of polyspermic fertilization.

The latest advance which has been accepted as the most successful micromanipulation technique is called the intracytoplasmic sperm injection (ICSI).In this technique, a single spermatozoon is mechanically inserted into the cytoplasm. In a mouse model, this technique has been first described by Lin et al (6) in 1966 and then has been used on humans but the results have been disappointing with high incidence of oocyte damage and low fertilization rate (7). Later in 1992 Palermo et al reported the first human pregnancies and live births (8). While performing a SUZI procedure at the Free University in Brussels one sperm accidentally penetrated the cytoplasm of an oocyte and resulted in fertilization. In the same institution, they performed ICSI to treat couples with infertility caused by severe male factor infertility and in whom IVF and SUZI procedures had failed. Of 47 metaphase II oocytes to which ICSI have been applied, 38 survived the procedure, 31 showed pronuclei and 15 embryos have been transferred to uterus. Four pregnancies occurred after 8 treatment cycles: Two singleton and two twin pregnancies and a miscarriage. Four healthy babies have been born after uneventful pregnancies. From the same center in a paper (9) comparing the SUZI and ICSI, they reported 91% and 18% survival and fertilization rates respectively through SUZI of 2214 oocytes while these rates have been 77% and 44% where a total of 716 oocytes have been manipulated through ICSI. Fourteen pregnancies have been achieved after transfer of 170 SUZI-treated embryos and 8 after 94 ICSI-treated embryos. In 1993, same group reported even a better experience with 87% and 51% survival and fertilization rates respectively (10). Recently, Cornell group reported a fertilization rate of 68.9% per intact oocyte and a clinical pregnancy rate of 41.7%, the overall ongoing pregnancy rate was 38.6% per oocyte retrieved (11).

The ICSI procedure: Same as in regular IVF cases, controlled ovarian hyperstimulation is achieved with exogenous gonadotropin therapy. As adjuvant agents, gonadotropin releasing hormone agonists (GnRH-a) are mostly used. When the oocytes reach the full size, with human chorionic gonadotropin administration their maturation are completed. At the same time of the oocyte retrieval, semen samples are

collected usually through masturbation. Depending on the count of the semen sample either epididymal sperm aspiration or testicular biopsy may be required. Sperm selection is performed by discontinuous Percoll gradient technique, subsequent washing, and concentration in culture medium.

Oocytes are collected through transvaginal route under ultrasound guidance 34-36 hours after the hCG injection. After the maturity grading, they are incubated approximately 4 hours. Then through hyaluronidase enzyme, the cumulus-corona cells are removed. Each oocyte is rinsed in fresh medium to remove residual enzyme and is examined under an inverted microscope to assess integrity and maturation stage. ICSI is performed only on metaphase II oocytes.

An inverted microscope equipped with two coarse-control manipulators and two micromanipulators are used to carry out the procedure. Before the injection, the sperm suspension is diluted with polyvinylpyrrolidone (PVP) solution and placed in the center of a Petri dish. This solution immobilizes the sperm, enhances the sperm handling and prevents the sperm from sticking to the pipette. Next, the oocytes are placed in single droplets of buffered medium surrounding the central droplet containing the sperm suspension. Two pipettes are used the holding pipette, which maintains the oocyte in place by suction on the zona pellucida at the 9 o'clock position, and the injection pipette. The single, apparently normal sperm chosen for the procedure is immobilized by touching its midpiece with the injection pipette and is aspirated tail first. The zona pellucida and oolemma are penetrated at 3 o'clock, the single sperm is released deeply into the cytoplasm, and the injection pipette is removed in one swift, gentle motion.

Oocytes are evaluated at 12 to 17 hours after the injection to determine the integrity, number and size of the pronuclei. Cleavage of fertilized oocytes is assessed 24 hours after fertilization. Approximately 72 hours after microinjection, up to four good quality embryos are transferred into the uterine cavity.

Indications of ICSI: Although there is no universally accepted criteria to use ICSI, it has been generally accepted to perform this procedure in extremely impaired male factor infertilities. A couple is considered unsuitable for conventional IVF if sperm concentration is less than 500.000 progressively motile spermatozoa in the entire ejaculate with a frequency of normal forms less than 3% (if strict criteria are applied). When the sperm count is $<5x10^6$/ml, through regular IVF the chance of pregnancy is extremely poor (12). Besides this, the fertilization failure after standard IVF cycles is the other main indication for ICSI.

New application fields for ICSI: Recently, in cases of male subfertility with surgically unreconstructable reproductive tract obstruction, direct microsurgical and percutaneous methods for sperm retrieval have been developed. To date, superior fertilization and clinical pregnancy rates have been reported when these recent sperm retrieval fails. The results of ICSI with epididymal spermatozoa are almost the same as with ejaculated sperm. In cases of obstructive azospermia where motile spermatozoa cannot be obtained from the epididymis it is possible to carry out ICSI using the sperm achieved through testicular biopsy (13). Spermatogenesis is normal in patients with obstructive azoospermia. In most of the non obstructive azospermic patients it is possible to find enough sperm to carry out the ICSI (14). In the literature, the best combination reported to date is microsurgical sperm aspiration (MESA) with ICSI. With this combination the fertilization and pregnancy rates are reported to be as high as 45% and 56% respectively (15).

Because of genetic or constitutional reasons, some men experience a maturational arrest of spermatogenesis, and the only haploid cell they produce is the round spermatid. Through ICSI these immature sperm cells can be injected to the oocytes. One group reported 4 pregnancies but they were all spontaneously aborted (16), so more clinical and cytogenetic studies are required in this new subject.

In some male factor infertility cases some abnormalities of the sperm other than the head may be seen. These abnormal regions of the sperm can be separated from the head through micromanipulation and only the head region can be injected to cytoplasm of the oocyte. This procedure is named as intracytoplasmic sperm head injection (ICSHI). Recently, Palermo et al (17) presented their experience of ICSHI on 35 Metaphase II oocytes; 89% survived and 74% were diploid (normal). They achieved a total of 16 (73%) embryos and transferred four of them back to the uterus together with some other ICSI-treated embryos. At the end, one twin pregnancy occurred.

In human genetics, X chromosome linked diseases constitute a very important place, so controlling the gender can prevent the spread of X-linked diseases in families at high risk. The studies to develop a 100% effective technique to separate the X and Y carrying spermatozoon before ICSI are still going on. Besides, after the fertilization for the purpose of early detection of the presence of the disease in the embryo, some preimplantation diagnostic techniques

are described through micromanipulation. They are alternative to prenatal diagnosis and therapeutic abortion.

Concerns about ICSI: The zone pellucida is the natural barrier to sperm penetration in mammalian eggs. In cases of semen problems like density, motility or morphology, micromanipulation procedures are used to bypass these natural obstacles to induce fertilization. It has been demonstrated that more morphological normal shaped spermatozoa bind and penetrate through the zona pellucida (18). It has also been shown that the normal ones bind also more to the oolemma. Thus it was concluded that the zona and oolemma act to select normal sperms. All these procedures bypassing the physiological events raise some concerns about the genetic safety of the ICSI procedure.

Specifically, in azospermic or severely oligozoospermic males through PCR the Y chromosome long arm has been analysed (19). They found that 10 out of 63 individuals studied, had deletions that potentially contributed to their disturbed spermatogenesis. Furthermore, the father of one of the patients has similar DNA deletions as his son. So there are concerns about the genetical evaluation of the embryos achieved through ICSI.

Reports of ICSI success using sperm aspirated from individuals with congenital bilateral absence of the vas deferens are emerging (20). It is well known that this anatomical defect as a result of a mutation is related to cystic fibrosis and occurs almost in 80% of cases (21). So in these cases preimplantation diagnosis should be applied to determie if a CF mutation has been passed on. A similar situation occurs in patients with Kartagener's syndrome (immotile cilia syndrome).

Another concern regarding the increased teratogenicity in ICSI procedure is the exposure of the oocyte to the PVP solution and its carcinogenic effect onto the later developing embryos. Recently in a study of sister-chromatid-exchange analysis it has been demonstrated that it did not have that effect (22).

In the Free University of Brussels they evaluated the obstetric outcome of 424 pregnancies after ICSI (23). They applied prenatal diagnosis and after the delivery followed the children. The genetist-pediatrician examinated these children at 2 and 12 months of age, and then once a year. So far they could not find any increased risk of congenital abnormality in children born through ICSI procedure. Similarly a group from Sweden evaluated their results concerning genetic abnormalities in 210 ICSI infants (24). Two major malformation occurred in 2 singleton children and 4 minor malformations occurred in 4 children. They found this rate within the range of expected values in Sweden and concluded that in the infants born after ICSI, the incidence of fetal abnormalities is no higher with ICSI than with standard IVF.

In conclusion there is now agreement among reproductive biologists that ICSI is the method of choice for treatment of a wide range of male infertility and fertilization dysfunction. Ongoing clinical work and basic research will provide answers to the questions surrounding the biological function of the ICSI. An important task for the future is to develop efficient methods for allocating patients to ICSI or standard IVF.

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