Reproductive BioMedicine Online (2010) **20**, 56–67

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REVIEW

Failed fertilization after clinical intracytoplasmic sperm injection

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Abstract Intracytoplasmic sperm injection (ICSI) has resulted in pregnancy and birth for many couples, including those with severe male factor infertility. However, even after ICSI, complete failure of fertilization occurs in 1–3% of cycles. Most cases occur due to low number of mature oocytes, failure of oocyte activation or non-availability of appropriate spermatozoa for injection. Given the significant emotional and financial involvement in assisted reproductive cycles, failure of fertilization in all mature oocytes is a distressful event. It is not predictable. Since follow-up ICSI cycles result in fertilization in 85% of cases, repeated ICSI attempts are suggested. Physicians should counsel patients experiencing repeated failure of fertilization after ICSI cycles about available options including donated oocytes/embryos, donor sperm insemination, adoption or remaining childless if these choices are not acceptable due to religious or ethical reasons. This review discusses the causes and remedies for failed fertilization after clinical ICSI. RBMOnline

KEYWORDS: failed fertilization; infertility; intracytoplasmic sperm injection; in-vitro fertilization

Introduction

When all other forms of assisted fertilization fail, intracytoplasmic sperm injection (ICSI) is the method of choice to overcome male factor infertility. The ICSI procedure allows direct injection of a single spermatozoon into the cytoplasm of an oocyte. Thus, fertilization is possible in cases in which sperm motility is impaired and inability to penetrate the zona pellucida is the major cause of infertility. ICSI is possible with spermatozoa obtained from ejaculation, microsurgical epididymal sperm aspiration, percutaneous epididymal sperm aspiration or testicular sperm extraction. In addition, indications for ICSI include idiopathic infertility and repeated conventional IVF failures (Benavida, et al., 1999).

Total failed fertilization (TFF) refers to failure of fertilization in all the mature oocytes and the term 'failed fertilization' refers to failure of fertilization in any mature oocyte. For all ages and with all the different sperm types,

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doi:10.1016/j.rbmo.2009.10.010

fertilization after ICSI is at about 70—80% (Palermo, et al., 2009). This suggests that, despite injecting spermatozoa into mature oocytes, failed fertilization still occurs. Given the considerable emotional and financial investment involved in a cycle of assisted reproduction, TFF is a distressing event for the infertile couple as well as the fertility professionals. TFF occurs in 5—10% of IVF cycles (Mahutte and Arici, 2003) and 1—3% of ICSI cycles (Flaherty, et al., 1998). TFF after ICSI cycles is mostly due to low number of mature oocytes (Flaherty et al., 1998) or oocyte activation failure (Ebner, et al., 2004). TFF is a rare event in cases with normal oocytes and spermatozoa (Mansour, et al., 2009). Some patients may face repeated TFF in spite of normal sperm parameters and good ovarian response (Tesarik, et al., 2002). In such cases, the primary reason for failed fertilization after ICSI is lack of oocyte activation, as more than 80% of these oocytes contain a spermatozoon (Flaherty et al., 1998). Considerable advances in artificial oocyte activation and recovery of spermatozoa from epididymis or testis that are suitable for ICSI help to avoid TFF. This review discusses the causes and remedies for failed fertilization after clinical ICSI.

Oocyte related factors

Oocyte morphology

Poor oocyte morphology is considered a major determinant of failed or impaired fertilization. Normal features of a healthy mature oocyte at metaphase II (MII) include: presence of a polar body, a round even shape, light colour cytoplasm with homogenous granularity, a small perivitelline space without debris and a colourless zona pellucida. In oocytes denuded for ICSI, the morphological structure and the nuclear maturity but not cytoplasmic maturity can be assessed in detail. The MII oocytes with apparently normal cytoplasmic organization may exhibit extracytoplasmic characteristics, such as increased perivitelline space, perivitelline debris and/or fragmentation of the first polar body, which have been suggested to reduce developmental competence of the oocyte involved (Xia, 1997). It is common for extra-cytoplasmic and cytoplasmic dysmorphisms to occur together in the same oocyte (**Figure 1**).

It has been suggested that dysmorphic phenotypes, which arise during meiotic maturation, may be associated with failed fertilization and aneuploidy, while those occurring later in maturation may cause a higher incidence of developmental [illegible] (Otsuki, et al., 2006; Van Blerkom and Henry, 2002).

Decreased fertilization rates with respect to some oocyte dysmorphisms have been reported (Xia, 1997), while others failed to observe that association (Ciotti, Nmarangelo, Morsclli-Labate, et al., 2004; De Santis, Cino, Rabellotti, et al., 2005; Meriano, Alexis, Visram-Zaver, et al., 2001; Mikkelsen and Lindenberg, 2001; Otsuki, et al., 2004). Meriano et al. (2001) reported lower pregnancy and implantation rates when the transferred embryos originated from cycles with more than 50% dysmorphic oocytes and the same dysmorphism repeated from one cycle to the other. The authors suggested that the repetitive organelle clustering was associated with an underlying adverse factor affecting the entire follicular cohort. The presence of a dark cytoplasm decreases the likelihood of obtaining good-quality embryos by 83% (Ten, Mendiola, Vioque, et al., 2007). However, an earlier study did not find any adverse impact of dark colour of the oocytes on the fertilization, embryo development and pregnancy rate (Esfandiari, Burjaq, Gotlieb, et al., 2006). In human oocytes, the cytoplasmic granularity can be homogeneous affecting the whole cytoplasm or concentrated in the centre with a clear peripheral ring giving a darkened appearance to the cytoplasm (Serhal, Ranieri, Kinis, et al., 1997). The abnormal changes in the cytoplasm of MII oocytes may be a reflection of delayed cytoplasmic maturation that is unsynchronized with nuclear maturity (Katz and Tur-Kaspa, 2006).

Normal fertilization, embryo development and births are achieved after ICSI in oocytes with dark zonae, abnormal morphology or repeated polyspermia following conventional IVF. The oocytes with extreme morphological abnormalities should not be discarded as ICSI may overcome the barriers to fertilization, cleavage and normal embryonic development (Esfandiari, Ryan, Gotlieb, et al., 2005b; Esfandiari et al., 2006). Zona-free oocytes may be fertilized normally after ICSI and develop to the blastocyst stage (Jelínková et al., 2001). Pregnancy in human (Stanger, Stevenson, Lakmaker, et al., 2007) and live birth in mouse (Naito, et al., 1992) and pig (Wu, Lai, Mao, et al., 2004) have been obtained after transfer of embryos resulting from zona-free oocytes.

Oocyte maturity

One of the major causes of TFF after ICSI is a low number of retrieved MII oocytes (Esfandiari, Javed, Gotlieb, et al., 2005a). About 20% of retrieved oocytes from ovarian stimulation cycles are immature, either at metaphase-I (MI) or germinal-vesicle (GV) stage in human IVF (Huang, Chang, Tsai, et al., 1999; Rienzi, Ubaldi, Anniballo, et al., 1998). Some of these oocytes may extrude the first polar body during in-vitro culture and may be used as a source of oocytes for sperm injection in ICSI cycles. However, the increase in the number of embryos derived from immature oocytes does not efficiently translate into pregnancies and live births. Therefore, the clinical significance of using immature oocytes in stimulated cycles needs further investigation (Shu, Gebhardt, Watt, et al., 2007).

The injection of MI oocytes immediately after denudation results in a high degeneration rate due to increased fragility of the oolemma. The fertilization rate of retrieved MI oocytes that remained MI at the time of ICSI is lower than the fertilization rate of retrieved sibling MI progressing to MII *in vitro* (25% compared with 62.2%, respectively). It is less than half when compared with the fertilization rate of retrieved sibling MII oocytes (69.5%). A high rate of multinucleated oocytes is also found in fertilized MI oocytes injected immediately after denudation (Shu et al., 2007).

In cases of poor responders and in patients with an unsynchronized cohort of follicles, where the presence of immature oocytes is frequent after stimulation (Smitz and Cortvrindt, 1999), the use of immature oocytes is important in order to increase the number of embryos obtained in each cycle. Based on the assumption that oocyte maturity is a prerequisite for obtaining normal fertilization, attempts