Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Should few retrieved oocytes be as an indication for intracytoplasmic sperm injection?

Qing-song XI, Li-xia ZHU[†‡], Juan HU, Li WU, Han-wang ZHANG

(*Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*)

[†]E-mail: zhulixiaxia@yahoo.com.cn

Received Dec. 13, 2011; Revision accepted June 29, 2012; Crosschecked July 31, 2012

Abstract: Objective: To reevaluate whether relatively few oocytes obtained in one cycle are an indication for intracytoplasmic sperm injection (ICSI). Methods: A total of 406 cycles with three or fewer retrieved oocytes performed in 396 non-male infertile couples were retrospectively reviewed. Cycles were classified into three groups by different fertilization techniques: the in vitro fertilization (IVF) group, insemination with conventional IVF; the ICSI group, insemination with ICSI though semen parameters were normal; and the rescue ICSI group, re-insemination with ICSI after conventional IVF failure. Results: The ICSI group resulted in higher normal fertilization compared with the conventional IVF group. Correspondingly, the cycle cancellation rate was decreased in the ICSI group, though it was not statistically significant. The clinical pregnancy rate and implantation rate were lower in the ICSI group compared with the conventional IVF group. Rescue ICSI was a method to avert total fertilization failure in conventional IVF, increasing fertilization and ensuring embryo availability for transfer, but the normal fertilization was the lowest due to delayed insemination and the chance of pregnancy was very little. Conclusions: Obtaining only few oocytes in one cycle is not considered as an indication for ICSI when the sperm sample is apparently normal. Rescue ICSI is either not recommended if conventional insemination fails. Such patients should not be subjected to the unnecessary costs and potential risks of ICSI.

Key words: Fertilization rate, Intracytoplasmic sperm injection (ICSI), Indication, Oocyte number, Patient age
doi:10.1631/jzus.B1100370 **Document code:** A **CLC number:** R714.13

1 Introduction

Since its first introduction in 1992, intracytoplasmic sperm injection (ICSI) has been applied worldwide to treat severe male factor infertility and fertilization dysfunction. Recently, however ICSI has been increasingly used in cases where conventional in vitro fertilization (IVF) would not necessarily be inefficient (Oehninger and Gosden, 2002; van der Westerlaken *et al*., 2005; Kim *et al*., 2007). The advantage of ICSI with non-male factor infertility is still the subject of debate. Staessen *et al.* (1999), Fishel *et al.* (2000), Plachot *et al.* (2002), Elizur *et al.* (2004), and van der Westerlaken *et al.* (2006) have stated that one advantage of ICSI is avoiding the risk of fertilization failure. However, many authors have reported no differences in terms of fertilization and implantation rates between ICSI and IVF (Bukulmez *et al.*, 2000; Kim *et al.*, 2007). In addition, some researches have indicated that ICSI is more efficient in terms of the number of fertilized oocytes and good quality embryos (Khamsi *et al.*, 2001).

The literature is also divided when a low number of oocytes are available for insemination. Some authors have found ICSI useful to achieve more fertilized oocytes and good quality embryos when the number of retrieved eggs is very low (Ludwig *et al.*, 1997) while others have reported no difference, even in patients with one (Gozlan *et al.*, 2007) or few (Borini *et al.*, 2009) oocytes inseminated.

So far, the management of these patients remains

‡ Corresponding author

controversial, and thus a number of patients and IVF centers have been reluctant to use ICSI as the first insemination choice because of its inconsistent outcomes.

It is important, therefore, to determine whether there is any therapeutic benefit to ICSI and whether it should be performed on patients when only few oocytes are available. With the improvement of insemination techniques and embryo culture environment, accompanied with the potential risks of ICSI to some extent unexplored, it is necessary to reevaluate whether relatively few obtained oocytes in one cycle are an indication for ICSI.

2 Subjects and methods

2.1 Patients

Given the expense, difficulty, and limitations, it is difficult to compare IVF and ICSI results in a prospective randomized trial. In our retrospective analysis, a cohort study was performed to investigate the prognosis of cycles with few retrieved oocytes in regards to the use of ICSI (rather than IVF). Four hundred and six cycles of 396 patients in which no more than three oocytes were retrieved were studied at the Reproductive Medicine Center of Tongji Hospital, Wuhan, China, from January 2009 to December 2010. All partners had normal semen parameters post-wash on the retrieval day. The patients were divided into three groups by different fertilization techniques: the IVF group, insemination with conventional IVF; the ICSI group, insemination with ICSI; and the rescue ICSI group, re-insemination after conventional IVF failure. To control for confounding variables, the cycle characteristics of each group were listed and compared.

2.2 Ovarian stimulation and sperm preparation

The patients were treated with gonadotropin-releasing hormone (GnRH) agonist (Decapeptyl, Ferring, Kiel, Germany; Diphereline Ipsen, Paris, France) from either the mid-luteal phase of the preceding cycle in a long treatment protocol or the second day of the cycle in a short treatment protocol. Ovarian stimulation was carried out with recombinant follicle-stimulating hormone (FSH) (Gonal-F, Serono, Aubonne, Switzerland). Follicular development was monitored by ultrasound scanning and serum hormone levels. Human chorionic gonadotropin (hCG 10000 IU) was administered when two or more follicles reached a mean diameter of 17–18 mm. Oocytes were retrieved by ultrasound-guided transvaginal puncture 36–38 h later.

Semen was collected in a sterile container by masturbation after 3–5 d of sexual abstinence and then kept for 30 min at 37 °C. After liquefaction, samples were analyzed for sperm concentration, motility, and morphology according to the World Health Organization (WHO) criteria. The semen samples were processed by the conventional swim-up method. If the sperm sample after preparation had a total motile sperm count (TMC) exceeding 2.0×10^6 with forward sperm motility ($a+b$)>50% and morphology (strict criteria) of >5% normal forms, the sperm were deemed normal.

2.3 Insemination

In the ICSI group, oocytes were treated with 0.5% (5 mg/ml) hyaluronidase (Sigma, St. Louis, Missouri, USA) for 10–20 s for enzymatic lysis of the cumulus oophorus cells. Cells of the corona radiate were removed mechanically with a Pasteur pipette under dissecting microscope guidance. Subsequently, the maturity of the oocytes was determined. Only oocytes in metaphase II were used for the ICSI procedure. The microinjection procedure was in accordance with van Steirteghem *et al.* (1993a; 1993b).

In the conventional IVF group, the oocytes were incubated in culture medium and inseminated with 5×10^4–10×10^4 motile spermatozoa 3–4 h after retrieval. Three hours later, the corona cells were removed to assess fertilization. If there was no sign of fertilization (no second polar body exhibited 6 h after insemination), rescue ICSI was then performed with the patient's consent.

Fertilization was confirmed by the observation of two pronuclei (PN) 14–18 h after IVF or ICSI. Cleavage was assessed the following day and good embryos of normal fertilization were then transferred.

2.4 Pregnancy

Clinical pregnancy was defined as the presence of a gestational sac as well as at least one fetal heartbeat on ultrasound screening. Abortion was defined as pregnancy loss before 20 weeks of gestational age.

2.5 Statistical analysis

The data were analyzed by chi-square test and $P<0.05$ was considered statistically significant.

3 Results

A total of 406 cycles performed in 396 patients were reviewed. The 406 cycles consisted of 297 in the IVF group, 72 in the ICSI group, and 37 in the rescue ICSI group (Table 1).

Table 1 Comparison of cycle characteristics and outcomes among groups

Parameter	Number		
	IVF	ICSI	Rescue ICSI
Cycles	297	72	37
Oocytes obtained	662	141	80
Retrieved oocytes	2.22±0.7	1.96±0.78	2.16±0.87
Metaphase II oocytes	568	121	73
Inseminated oocytes	1.23±0.93	1.30±0.80	1.16±0.90
Normal fertilization	364 (64.1%)	94 (77.7%)*	43 (58.9%)#
Abnormal fertilization	84 (14.8%)	10 (8.3%)	7 (9.6%)
Cycles with embryo transfer	230 (77.4%)	62 (86.1%)	26 (70.3%)#
Cancelled cycles	67	10	11
Embryos transferred	354	93	38
Pregnancy per transferred cycles	57 (24.8%)	6 (9.7%)*	2 (7.7%)
Pregnancy per initiated cycles	57 (19.2%)	6 (8.3%)*	2 (5.4%)
Implantation	65 (18.4%)	6 (6.5%)*	2 (5.3%)
Abortion	8 (14.0%)	1 (16.7%)	1 (50.0%)

Age of female: (34.5±4.6) years in the IVF group; (36.1±5.5) years in the ICSI group; (34.7±5.4) years in the rescue ICSI group. Values are expressed as mean±SD, *n*, or *n* (%). * $P<0.01$ as compared with the IVF group; # $P<0.01$ as compared with the ICSI group

The normal fertilization rate was dramatically decreased in the IVF group compared with the ICSI group (64.1% vs. 77.7% (364/568 vs. 94/121), $P=0.004$), while the abnormal fertilization (1PN and 3PN) rate was increased in the IVF group compared with the ICSI group (14.8% vs. 8.3% (84/568 vs. 10/121), $P=0.058$), though this was not significantly different. Hence, more couples in the ICSI group had the chance of embryo transfer. The percentage of cycles with transfer was 77.4% (230/297) in the IVF group compared with 86.1% (62/72) in the ICSI group ($P=0.104$).

The increased number of embryos for transfer in the ICSI group did not result in an improved pregnancy rate. The pregnancy rate in the ICSI group was actually lower than that in the IVF group, and the implantation rate was decreased as well.

If conventional IVF failed, rescue ICSI was a useful way to re-inseminate to avert cycle cancellation, though it could not avert cycle cancellation completely. The normal fertilization and embryos available were relatively low compared with the conventional ICSI group due to delayed insemination and oocyte aging. Of the 37 cycles rescued, 26 cycles had the chance of embryo transfer. Only two couples became pregnant, but one ended in spontaneous abortion.

Table 2 shows results for a specified group of patients of the reviewed cycles who were aged 40 years and over. No significant differences were found between the IVF and ICSI groups in terms of normal fertilization, abnormal fertilization, and cycle cancellation rate. Seven couples achieved pregnancy in the IVF group. The pregnancy rate was 18.9% (7/37). Of the 22 cycles with embryo transfer, no pregnancy was achieved in the ICSI group of the specified patients.

Table 2 Comparison of outcomes between the IVF group and ICSI group in female patients aged 40 years and over

Parameter	Number	
	IVF	ICSI
Cycles	44	27
Oocytes obtained	92	48
Retrieved oocytes	1.91±0.86	2.07±0.78
Metaphase II oocytes	78	45
Inseminated oocytes	1.18±0.90	1.33±0.83
Normal fertilization	55 (70.5%)	33 (73.3%)
Abnormal fertilization	7 (9.0%)	5 (11.1%)
Cycles with embryo transfer	37 (84.1%)	22 (81.5%)
Embryos transferred	56	33
Pregnancy per transferred cycles	7 (18.9%)	0
Pregnancy per initiated cycles	7 (15.9%)	0
Implantation	7 (12.5%)	0

Age of female: (41.7±1.8) years in the IVF group; (42.4±2.4) years in the ICSI group. Values are expressed as mean±SD, *n*, or *n* (%)

4 Discussion

ICSI was first applied for cases of severe male factor infertility, where conventional IVF was expected to fail or give poor outcomes. Some recent reports have shown that ICSI (one egg, one spermatozoon) can be used successfully on non-male factor couples to provide comparable or higher fertilization rates (Palermo *et al*., 1996; Khamsi *et al*., 2001) and superior embryo quality (Yang *et al*., 1996) than conventional IVF. Thus, ICSI has been increasingly used in cases in which conventional IVF would not necessarily be inefficient, especially when there are few oocytes available.

Unlike ICSI, in which one sperm is inserted into the cytoplasm of an oocyte, in conventional IVF an oocyte is exposed to 5×10^4–10×10^4 motile sperm. There is the possibility that more than one sperm could enter the oocyte, which might increase the risk of poly-pronuclei zygotes, and thereby decrease the number of available embryos. Therefore, most assisted reproductive techniques (ART) follow a policy of performing ICSI in cases where few oocytes are available. These oocytes are considered 'extremely valuable', and therefore the use of ICSI may result in higher fertilization rates and thereby improve the chance of pregnancy accordingly.

Our retrospective analysis showed that when ICSI was used to treat non-male infertile couples, greater rates of normal fertilization and numbers of embryos were observed, but it resulted in lower pregnancy and implantation rates. This may relate to the expression of paternal genes that begin at the 4–8 cell stages. It is only at this stage that the consequences of paternal DNA-induced alterations may manifest and impair embryo development (Borini *et al.*, 2006). For this reason the forced introduction of sperm into the oocyte by ICSI may lead to higher numbers of fertilized oocytes and embryos available for transfer with respect to IVF, but may not correlate with the implantation rate.

Since the number of available oocytes is usually associated with physiologic aging and ovary response (Abdalla *et al*., 1993; Navot *et al*., 1994), ICSI is more prone to be applied in patients of advanced maternal age who are poor responders independent of semen quality. In order to omit this age bias, we further analyzed the results in the original study group in relation to females aged 40 years and over. No pregnancy was obtained in the ICSI group for these specific patients. The results also indicated that there was no advantage for an infertile couple undergoing ICSI with an apparently normal sperm sample, even if the patient was of advanced maternal age. These results are consistent with those of Borini *et al.* (2009).

The higher pregnancy and implantation rate after IVF may be explained by a kind of 'natural sperm selection'. On one hand, natural selection may act against chromosomally abnormal oocytes. Chromosomal analysis of failed fertilized oocytes after IVF has shown that about one third of the oocytes had an abnormal karyotype (Kunathikom *et al*., 2001). ICSI is an invasive procedure that bypasses most steps of the fertilization process. Performing ICSI may result in some intrinsically or developmentally defective oocytes being fertilized and forming an embryo, which may lead in no pregnancy or pregnancy with abortion. On the other hand, natural selection may also act against subtly defective sperm. In ICSI operation, scientists always attempt to select morphologically normal motile sperm for ICSI, such a subjective method is often inconsistent and it is impossible to distinguish sperm with or without fragmented or denatured DNA on fresh motile sperm.

In addition to the conflicting data surrounding the use of different insemination techniques in cycles with few oocytes available, there are other factors to consider.

Taking cost into consideration, ICSI is a more expensive process (Ola *et al*., 2001). The application of ICSI usually results in more fertilization and available embryos, and therefore more infertile couples have the chance of embryo transfer. However, the pregnancy and implantation rates are not improved with the ICSI procedure. Thus, infertile couples endure significant economic and emotional pressure in the pursuit of pregnancy without an associated increase in the rate of pregnancy. In addition, ICSI is a sophisticated operating procedure, which is a more time-consuming and complex than conventional IVF (Taylor *et al*., 2008), and therefore increases the workload of staff at fertility clinics and centers.

Another concern is the invasiveness to the gametes (Plachot *et al*., 2002). Not only is the ICSI procedure itself an invasiveness process, the ICSI

oocytes may also be associated with an increased risk of genetic defects in offspring. During the operation procedure, non-biological materials such as media or polyvinylpyrrolidone (PVP) might be injected into the oocytes. Moreover, the ICSI needle itself, which can disturb the chromosomes on the meiotic spindle, may increase potential genetic defects (Hardarson *et al*., 2000; Woldringh *et al*., 2005), though this needs further study. Research also suggests that ICSI can disrupt oocyte activation and lead to failed fertilization (Flaherty *et al*., 1995). Recent studies have also reported an increase in sex chromosomal anomalies in ICSI offspring, although they are exceedingly rare (Bonduelle *et al*., 2002).

Rescue ICSI, which is a newly introduced method to avert total fertilization failure, has been increasingly applied worldwide with highly praised results. However, this process does not adapt to all cases of total fertilization failure. In our study, 37 cycles were rescued by means of re-insemination, with 26 cycles obtaining the chance of transfer. However, only one achieved an on-going pregnancy. Oocyte aging because of delayed insemination may be one of the reasons for such a poor outcome (Zhu *et al*., 2011a; 2011b).

A constant pursuit of reproductive medicine is to find and apply the simplest, most economical, and least invasive method to give infertile couples the best chance of conceiving a healthy baby.

As ICSI is such a complex procedure, concerns remain regarding its safety. Based on our research, patients with few retrieved oocytes and normal semen parameters received no benefit from ICSI and need not be subjected to its high cost and potential risks, even if the female was at an advanced age. When fertilization failure occurred in these cases, it was likely the result of natural selection, and thus rescue ICSI to re-inseminate is not recommended.

References

Abdalla, H.I., Burton, G., Kirkland, A., Johnson, M.R., Leonard, T., Brooks, A.A., Studd, J.W., 1993. Age, pregnancy and miscarriage: uterine versus ovarian factor. *Hum. Reprod*., **8**(9):1512-1517.

Bonduelle, M., van Assche, E., Joris, H., Keymolen, K., Devroey, P., van Steirteghem, A., Liebaers, I., 2002. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum. Reprod*., **17**(10):2600-2614. [doi:10.1093/humrep/17.10.2600]

Borini, A., Tarozzi, N., Bizzaro, D., Bonu, M.A., Fava, L., Flamigni, C., Coticchio, G., 2006. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. *Hum. Reprod*., **21**(11):2876-2881. [doi:10.1093/humrep/del251]

Borini, A., Gambardella, A., Bonu, M.A., Dal Prato, L., Sciajno, R., Bianchi, L., Cattoli, M., 2009. Comparison of IVF and ICSI when only few oocytes are available for insemination. *Reprod. Biomed. Online*, **19**(2):270-275. [doi:10.1016/S1472-6483(10)60084-3]

Bukulmez, O., Yarali, H., Yucel, A., Sari, T., Gurgan, T., 2000. Intracytoplasmic sperm injection versus in vitro fertilization for patients with a tubal factor as their sole cause of infertility: a prospective, randomized trial. *Fertil. Steril*., **73**(1):38-42. [doi:10.1016/S0015-0282(99)00449-5]

Elizur, S.E., Levron, J., Seidman, D.S., Kees, S., Levran, D., Dor, J., 2004. Conventional in vitro fertilization versus intracytoplasmic sperm injection for sibling oocytes in couples with mild oligoteratoasthenozoospermia and couples with normal sperm. *Fertil. Steril*., **82**(1):241-243. [doi:10.1016/j.fertnstert.2003.11.053]

Fishel, S., Aslam, I., Lisi, F., Rinaldi, L., Timson, J., Jacobson, M., Gobetz, L., Green, S., Campbell, A., Lisi, R., 2000. Should ICSI be the treatment of choice for all cases of in-vitro conception? *Hum. Reprod*., **15**(6):1278-1283. [doi:10.1093/humrep/15.6.1278]

Flaherty, S.P., Payne, D., Swann, N.J., Matthews, C.D., 1995. Aetiology of failed and abnormal fertilization after intracytoplasmic sperm injection. *Hum. Reprod*., **10**(10):2623-2636.

Gozlan, I., Dor, A., Farber, B., Meirow, D., Feinstein, S., Levron, J., 2007. Comparing intracytoplasmic sperm injection and in vitro fertilization in patients with single oocyte retrieval. *Fertil. Steril*., **87**(3):515-518. [doi:10.1016/j.fertnstert.2006.07.1515]

Hardarson, T., Lundin, K., Hamberger, L., 2000. The position of the metaphase II spindle can not be predicted by the location of the first polar body in the human oocyte. *Hum. Reprod*., **15**(6):1372-1376. [doi:10.1093/humrep/15.6.1372]

Khamsi, F., Yavas, Y., Roberge, S., Wong, J.C., Lacanna, I.C., Endman, M., 2001. Intracytoplasmic sperm injection increased fertilization and good-quality embryo formation in patients with non-male factor indications for in vitro fertilization: a prospective randomized study. *Fertil. Steril*., **75**(2):342-347. [doi:10.1016/S0015-0282(00)01674-5]

Kim, H.H., Bundorf, M.K., Behr, B., McCallum, S.W., 2007. Use and outcomes of intracytoplasmic sperm injection for non-male factor infertility. *Fertil. Steril*., **88**(3):622-628. [doi:10.1016/j.fertnstert.2006.12.013]

Kunathikom, S., Makemaharn, O., Suksompong, S., Laokirkkiat, P., 2001. Chromosomal analysis of "failed-fertilized" human oocytes resulting from in-vitro fertilization and intracytoplasmic sperm injection. *J. Med. Assoc. Thai*, **84**(4):532-538.

Ludwig, M., al-Hasani, S., Küpker, W., Bauer, O., Diedrich, K., 1997. A new indication for an intracytoplasmic sperm injection procedure outside the cases of severe male factor infertility. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **75**(2):207-210. [doi:10.1016/S0301-2115(97)00128-0]

Navot, D., Drews, M.R., Bergh, P.A., Guzman, I., Karstaedt, A., Scott, R.T.Jr., Garrisi, G.J., Hofmann, G.E., 1994. Age-related decline in female fertility is not due to diminished capacity of the uterus to sustain embryo implantation. *Fertil. Steril.*, **61**(1):97-101.

Oehninger, S., Gosden, R.G., 2002. Should ICSI be the treatment of choice for all cases of in-vitro conception? No, not in light of the scientific data. *Hum. Reprod.*, **17**(9):2237-2242. [doi:10.1093/humrep/17.9.2237]

Ola, B., Afnan, M., Sharif, K., Papaioannou, S., Hammadieh, N., Barratt, C.L., 2001. Should ICSI be the treatment of choice for all cases of in-vitro conception? Considerations of fertilization and embryo development, cost effectiveness and safety. *Hum. Reprod.*, **16**(12):2485-2490. [doi:10.1093/humrep/16.12.2485]

Palermo, G.D., Cohen, J., Rosenwaks, Z., 1996. Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. *Fertil. Steril.*, **65**(5):899-908.

Plachot, M., Belaisch-Allart, J., Mayenga, J.M., Chouraqui, A., Tesquier, L., Serkine, A.M., 2002. Outcome of conventional IVF and ICSI on sibling oocytes in mild male factor infertility. *Hum. Reprod.*, **17**(2):362-369. [doi:10.1093/humrep/17.2.362]

Staessen, C., Camus, M., Clasen, K., de Vos, A., van Steirteghem, A., 1999. Conventional in-vitro fertilization versus intracytoplasmic sperm injection in sibling oocytes from couples with tubal infertility and normozoospermic semen. *Hum. Reprod.*, **14**(10):2474-2479. [doi:10.1093/humrep/14.10.2474]

Taylor, T.H., Wright, G., Jones-Colon, S., Mitchell-Leef, D., Kort, H.I., Nagy, Z.P., 2008. Comparison of ICSI and conventional IVF in patients with increased oocyte immaturity. *Reprod. Biomed. Online*, **17**(1):46-52. [doi:10.1016/S1472-6483(10)60292-1]

van der Westerlaken, L., Helmerhorst, F., Dieben, S., Naaktgeboren, N., 2005. Intracytoplasmic sperm injection as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization. *Fertil. Steril.*, **83**(3):612-617. [doi:10.1016/j.fertnstert.2004.08.029]

van der Westerlaken, L., Naaktgeboren, N., Verburg, H., Dieben, S., Helmerhorst, F.M., 2006. Conventional in vitro fertilization versus intracytoplasmic sperm injection in patients with borderline semen: a randomized study using sibling oocytes. *Fertil. Steril.*, **85**(2):395-400. [doi:10.1016/j.fertnstert.2005.05.077]

van Steirteghem, A.C., Liu, J., Joris, H., Nagy, Z., Janssenswillen, C., Tournaye, H., Derde, M.P., van Assche, E., Devroey, P., 1993a. Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. *Hum. Reprod.*, **8**(7):1055-1060.

van Steirteghem, A.C., Nagy, Z., Joris, H., Liu, J., Staessen, C., Smitz, J., Wisanto, A., Devroey, P., 1993b. High fertilization and implantations rates after intracytoplasmic sperm injection. *Hum. Reprod.*, **8**(7):1061-1066.

Woldringh, G.H., Krcnicr, J.A., Braat, D.D., Meuleman, E.J., 2005. Intracytoplasmic sperm injection: a review of risks and complications. *BJU Int.*, **96**(6):749-753. [doi:10.1111/j.1464-410X.2005.05708.x]

Yang, D., Shahata, M.A., Al-Bader, M., Al-Natsha, S.D., Al-Flamerzia, M., Al-Shawaf, T., 1996. Intracytoplasmatic sperm injection improving embryo quality: comparison of the sibling oocytes of non-male-factors couples. *J. Assist. Reprod. Genet.*, **13**(4):351-355. [doi:10.1007/BF02070151]

Zhu, L., Ren, X., Wu, L., Hu, J., Li, Y., Zhang, H., Xi, Q., 2011a. Rescue ICSI: choose the optimal rescue window before oocyte aging. *J. Reprod. Contracept.*, **22**(1):29-36. [doi:10.1016/S1001-7844(12)60004-2]

Zhu, L., Xi, Q., Nie, R., Chen, W., Zhang, H., Li, Y., 2011b. Rescue intracytoplasmic sperm injection: a prospective randomized study. *J. Reprod. Med.*, **56**(9-10):410-414.