

# Intracytoplasmic sperm injection (ICSI) for non-male factor infertility: a committee opinion

The Practice Committees of the American Society for Reproductive Medicine and Society for Assisted Reproductive Technology

American Society for Reproductive Medicine, Birmingham, Alabama

Intracytoplasmic sperm injection, while typically effective for overcoming low or absent fertilization in couples with a clear abnormality of semen parameters, is frequently utilized in combination with assisted reproductive technologies for other etiologies of infertility in the presence of normal semen parameters. This committee opinion provides a critical review of the literature, where available, to identify situations where this may or may not be of benefit. (Fertil Steril® 2012;98:1395–9. ©2012 by American Society for Reproductive Medicine.)

Earn online CME credit related to this document at [www.asrm.org/learn](http://www.asrm.org/learn)

**Discuss:** You can discuss this article with its authors and with other ASRM members at <http://fertstertforum.com/goldsteinintracytoplasmic-sperm-injection-for-non-male-factor/>



Use your smartphone to scan this QR code and connect to the discussion forum for this article now.\*

\* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Intracytoplasmic sperm injection (ICSI) was introduced in 1992 to improve fertilization in couples with male factor infertility undergoing in vitro fertilization (IVF) or in couples with fertilization failure in a prior IVF cycle without detectable abnormalities of semen parameters (1–3). While the diagnostic criteria used to identify male factor infertility fail to predict with perfect accuracy poor or absent fertilization in assisted reproductive technology (ART) (4–7), studies to date support the safety and efficacy of ICSI to treat various male factor conditions.

The use of ICSI for patients with borderline or even normal semen parameters has become more common (8, 9). Proposed indications for use of ICSI include: unexplained infertility, poor-quality oocytes, low oocyte yield, advanced maternal age, prior fertilization failure with conventional insemination, routine use in all IVF

cycles, preimplantation genetic testing (PGT), fertilization after in vitro maturation (IVM), and fertilization of cryopreserved oocytes. The rationale for all these indications, with the exception of PGT, is avoiding fertilization failure. When using ICSI in these settings, the likelihood of fertilization failure must be balanced against any potential risks of the procedure and its costs.

## ICSI FOR UNEXPLAINED INFERTILITY

ICSI has been proposed for use in patients with unexplained infertility, since its use may bypass potential fertilization barriers that could be the cause of the unexplained infertility. Two studies in patients with unexplained infertility compared conventional insemination with ICSI using sibling oocytes. Fertilization rates after ICSI, even

when the immature oocytes not subjected to ICSI were included, were higher than the conventionally inseminated group: 65.3% vs. 48.1%,  $P < .001$  and 61.0% vs. 51.6%,  $P < .001$  for the two studies respectively (10, 11). Fertilization failure occurred more commonly in the conventional insemination groups than in the ICSI groups: 0% vs. 16.7%,  $P < .002$  and 0.8% vs. 19.2%,  $P < .001$  respectively (10, 11). Other studies have confirmed these findings (12–16). However, since these studies used sibling oocytes and the embryos transferred were a mixture from the inseminated and ICSI groups, no information about the effect of insemination or ICSI on clinical outcomes such as implantation, pregnancy, or live-birth rates could be ascertained from these studies.

A study of 60 women with unexplained infertility randomized patients to IVF with conventional insemination or ICSI (17). The study found no significant differences in the primary outcome (fertilization rate 77.2% vs. 82.4%) or in secondary outcomes: embryo quality, implantation rate (38.2% vs. 44.4%), clinical pregnancy rate (50% in each group), or live-birth rate

Received August 13, 2012; accepted August 14, 2012; published online September 12, 2012.

No reprints will be available.

Correspondence: Practice Committee, American Society for Reproductive Medicine, 1209 Montgomery Hwy., Birmingham, AL 35216 (E-mail: [ASRM@asrm.org](mailto:ASRM@asrm.org)).

Fertility and Sterility® Vol. 98, No. 6, December 2012 0015-0282/\$36.00

Copyright ©2012 American Society for Reproductive Medicine, Published by Elsevier Inc. <http://dx.doi.org/10.1016/j.fertnstert.2012.08.026>

(46.7% vs. 50%). There were two cases of failed fertilization in the conventional insemination group. The study was limited, however, by its small sample size. Similarly, another randomized trial comparing conventional insemination to ICSI in 100 couples with unexplained infertility revealed no difference in pregnancy rates between the two treatment groups (IVF 32%, ICSI 38%; relative risk (RR) 0.83 [95% confidence interval (CI) 0.48–1.45]) (18). Fertilization failure occurred in only one couple (out of 48) in the conventional insemination group.

Overall, the current evidence regarding the routine use of ICSI for unexplained infertility is limited and does not demonstrate improvement in clinical outcomes. Further studies are thus needed to determine the role of ICSI in this population.

### ICSI FOR POOR-QUALITY OOCYTES

Morphologically abnormal oocytes (with either nuclear, cytoplasmic, or zona pellucida abnormalities) in the presence of normal semen parameters create a clinical challenge. No studies addressing whether the use of ICSI in such cases improves clinical outcomes were identified for this document.

### ICSI FOR LOW OOCYTE YIELD

ICSI is commonly used in cases of low oocyte yield, in theory to increase the number of embryos achieved compared to that expected with conventional insemination. One controlled trial randomized 96 patients without male factor who had six or fewer oocytes to ICSI or conventional insemination (19). When comparing ICSI and conventional insemination, mean ages of the patients (35.3 and 36.7 years, respectively) and mean number of oocytes retrieved (4.4 and 4.5 oocytes, respectively) were similar. ICSI provided statistically similar outcomes compared to conventional insemination in terms of fertilization rates (77.7% vs. 70.2%), fertilization failure (11.5% vs. 11.5%), embryo quality, mean embryos per patient (2.5 vs. 2.2), clinical pregnancy rates (17.3% vs. 21.1%), and miscarriage rates (33.3% vs. 36.4%). A recent large retrospective analysis confirmed these findings (20).

Based on current evidence, the use of ICSI for low oocyte yield does not significantly improve fertilization rates, embryo number and quality, or pregnancy rates.

### ICSI FOR ADVANCED MATERNAL AGE

Oocytes retrieved from older women have been theorized to have structural defects of the zona pellucida or cytoplasm that might reduce the fertilization rate with conventional insemination. In practice, oocyte fertilization rates in women over 35 years of age using conventional insemination are similar to fertilization rates in younger women (16). No studies assessing the benefits of ICSI in this specific group of patients for any outcomes such as embryo quality or implantation rate were identified for this document.

### ICSI FOR PRIOR FAILED FERTILIZATION WITH CONVENTIONAL INSEMINATION

The use of ICSI in IVF following prior total failed fertilization with normal semen analysis in a prior IVF cycle is

advocated to reduce the risk of subsequent failed fertilization. Retrospective studies have shown that in cycles where there was total fertilization failure in IVF/conventional insemination, subsequent fertilization rates using IVF/conventional insemination again ranged from 30–97% (21–23). Subsequent total failed fertilization was correlated with number of follicles, oocytes retrieved, and mature oocytes. In a prospective study, sister oocytes were allocated to conventional insemination vs. ICSI in the IVF cycle following total failed fertilization with IVF/conventional insemination (24). In this study subsequent conventional insemination resulted in 12/109 (11%) oocytes fertilized by IVF/conventional insemination and 78/162 (48%) fertilized with IVF/ICSI. Although subsequent total failed fertilization may be related to quality of the IVF stimulation, utilizing IVF/ICSI may decrease the risk of subsequent poor fertilization failure.

### ICSI FOR ROUTINE USE

The routine use of ICSI for all oocytes, regardless of the etiology of the infertility, has been proposed (25, 26). The rationale is to reduce the likelihood of fertilization failure and potentially increase the number of embryos. A well-powered multi-center, randomized, controlled trial compared outcomes after conventional insemination or ICSI in 415 couples with non-male factor infertility (27). The fertilization rate per oocyte retrieved was higher with conventional insemination than with ICSI (58% vs. 47%,  $P < .0001$ ). Fertilization failure occurred in 5% (11/206) and 2% (4/209) in the conventional insemination and ICSI groups, respectively. Based on these data, the number needed to treat (NNT) with ICSI to prevent one case of fertilization failure with conventional insemination is 33. Additionally, this study reported similar clinical pregnancy rates with conventional insemination and ICSI (33% vs. 26%, RR 1.27 [95% CI 0.95–1.72]). The study concluded that use of ICSI should be reserved only for male factor infertility. Other non-randomized studies comparing conventional insemination to routine ICSI have found no significant differences in fertilization rate, failed fertilization, clinical pregnancy rates, or live-birth rates (28–31). Although the risk of failed fertilization is low, it occurs with similar frequency following both conventional insemination and ICSI. The emotional and financial costs of failed fertilization must be taken into consideration.

The routine use of ICSI for all oocytes does not appear to be justified in cases without male factor infertility or a history of prior fertilization failure based on available evidence.

### ICSI FOR PGT

ICSI is used in cases requiring PGT of embryos. The rationale for ICSI use is to ensure monospermic fertilization and eliminate potential paternal contamination from extraneous sperm attached to the zona pellucida (32, 33). While there are no randomized, controlled trials, the concerns of inaccurate results due to extraneous sperm contamination with PGT justifies the use of ICSI in this situation.

## ICSI AFTER IVM

The investigational process of IVM may lead to alterations in the zona pellucida, which reduce the fertilization potential of oocytes using conventional insemination (34, 35). One study randomly assigned mature oocytes after being denuded of their cumulus cells to conventional insemination or ICSI. Oocytes that were allowed to mature in vitro with or without their cumulus complexes had significantly lower fertilization rates with conventional insemination compared to ICSI (56.3% vs. 84.1%,  $P < .01$  and 39.5% vs. 84.5%,  $P < .01$ , respectively) (34). An additional study similarly demonstrated conventional insemination fertilization rates of 37.7% of mature oocytes (although oocyte maturity was not assessed until the fertilization check 18 hours after insemination), compared to a 69.3% fertilization rate using ICSI of metaphase II oocytes. While pregnancy rates were similar between the conventional insemination and ICSI groups (23.8% and 17.1%, respectively,  $P =$  not significant), the implantation of oocytes fertilized with standard insemination techniques was higher than those where ICSI was used (24.2% vs. 14.8%,  $P < .05$ ) (25). While ICSI may improve fertilization rates of in vitro matured oocytes, further studies are needed to evaluate this hypothesis.

## ICSI FOR CRYOPRESERVED OOCYTES

In general, oocyte cryopreservation involves the removal of the cumulus cells prior to freezing. This may lead to changes in the zona pellucida that could reduce fertilization rates with conventional insemination. For these reasons, ICSI has been the preferred method of fertilizing cryopreserved oocytes. Limited data exist comparing conventional insemination to ICSI for cryopreserved oocytes (36).

## OTHER CONSIDERATIONS OF ICSI FOR NON-MALE FACTOR INFERTILITY

The safety of ICSI for non-male factor infertility has not been evaluated. However, in studies of male factor infertility, ICSI has been associated with a small increased risk of adverse outcomes in offspring. These risks are generally attributed to the underlying male factor. It is unknown how these risks may relate to ICSI for non-male factor patients (37–46).

One large population cohort study including over 308,000 births, with over 6100 from ART, noted that the risk of major birth defects after IVF (with or without ICSI) had an odds ratio of 1.2 (95% CI, 1.09 to 1.41) after adjustment for several potential confounders (47). When the women undergoing IVF alone were separated from those also undergoing ICSI, only those undergoing ICSI still had an increased odds ratio for birth defects (1.57; 95% CI, 1.30 to 1.90). However, this study included men with and without normal sperm counts. The increased rate of birth defects after IVF in men with abnormal semen analyses is well recognized, given the known chromosomal abnormalities in such men, and is not unexpected in this study. Still, this study injects an additional note of caution into the indiscriminate use of ICSI in all IVF cycles.

ICSI requires additional laboratory experience, resources, effort, and time. Thus, expanded use of ICSI increases the complexity and cost of IVF.

## SUMMARY

- ICSI is a safe and effective therapy for the treatment of male factor infertility.
- ICSI can increase fertilization rates when lower than expected or failed fertilization has previously occurred with conventional insemination.
- ICSI for unexplained infertility does not improve clinical outcomes.
- ICSI for low oocyte yield and advanced maternal age does not improve clinical outcomes.
- ICSI may improve fertilization rates in a subsequent cycle following total failed fertilization in a prior IVF/conventional insemination cycle, although fertilization failure seems to correlate with poor ovarian stimulation.
- ICSI for routine use may decrease the incidence of unexpected failed fertilization; however, more than 30 couples would have to undergo ICSI unnecessarily to prevent one failed fertilization.
- ICSI may be of benefit for patients undergoing IVF with PGT, in vitro matured oocytes, and previously cryopreserved oocytes.

## CONCLUSIONS

- There are no data to support the routine use of ICSI for non-male factor infertility.
- ICSI may be beneficial for patients using PGT, IVM, or cryopreserved oocytes.
- The safety and cost of ICSI in the setting of non-male factor infertility must be considered.

**Acknowledgments:** This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine (ASRM) in collaboration with the Society for Assisted Reproductive Technology (SART) as a service to its members and other practicing clinicians. Although this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committees and the Board of Directors of ASRM and SART have approved this report.

The following members of the ASRM Practice Committee participated in the development of this document. All Committee members disclosed commercial and financial relationships with manufacturers or distributors of goods or services used to treat patients. Members of the Committee who were found to have conflicts of interest based on the relationships disclosed did not participate in the discussion or development of this document.

Samantha Pfeifer, M.D.; Marc Fritz, M.D.; Jeffrey Goldberg, M.D.; R. Dale McClure, M.D.; Roger Lobo, M.D.; Michael Thomas, M.D.; Eric Widra, M.D.; Glenn Schattman, M.D.; Mark Licht, M.D.; John Collins, M.D.; Marcelle Cedars, M.D.; Catherine Racowsky, Ph.D.; Michael Vernon, Ph.D.; Owen Davis, M.D.; Kurt Barnhart, M.D., M.S.C.E.; Clarisa Gracia, M.D., M.S.C.E.; Kim Thornton, M.D.; William Catherino, M.D., Ph.D.; Robert Rebar, M.D.; Andrew La Barbera, Ph.D.

## REFERENCES

- Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1999; 340:17-8.
- Benadiva CA, Nulsen J, Siano L, Jennings J, Givargis HB, Maier D. Intracytoplasmic sperm injection overcomes previous fertilization failure with conventional in vitro fertilization. *Fertil Steril* 1999;72:1041-4.
- Kastrop PM, Weima SM, Van Kooij RJ, Te Velde ER. Comparison between intracytoplasmic sperm injection and in-vitro fertilization (IVF) with high insemination concentration after total fertilization failure in a previous IVF attempt. *Hum Reprod* 1999;14:65-9.
- Genetic considerations related to intracytoplasmic sperm injection (ICSI). *Fertil Steril* 2006;86:103-5.
- Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, et al. Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med* 2001;345:1388-93.
- Tournaye H, Verheyen G, Albano C, Camus M, Van Landuyt L, et al. Intracytoplasmic sperm injection versus in vitro fertilization: a randomized controlled trial and a meta-analysis of the literature. *Fertil Steril* 2002;78:1030-7.
- van Rumste MM, Evers JL, Farquhar CM. Intra-cytoplasmic sperm injection versus conventional techniques for oocyte insemination during in vitro fertilisation in patients with non-male subfertility. *Cochrane Database Syst Rev* 2003;CD001301.
- Jain T, Gupta RS. Trends in the use of intracytoplasmic sperm injection in the United States. *N Engl J Med* 2007;357:251-7.
- American Society for Reproductive Medicine. Intracytoplasmic sperm injection (ICSI). *Fertil Steril* 2008;90:S187.
- Hershlag A, Paine T, Kvapil G, Feng H, Napolitano B. In vitro fertilization-intracytoplasmic sperm injection split: an insemination method to prevent fertilization failure. *Fertil Steril* 2002;77:229-32.
- Jaroudi K, Al-Hassan S, Al-Sufayan H, Al-Mayman H, Qeba M, Coskun S. Intracytoplasmic sperm injection and conventional in vitro fertilization are complementary techniques in management of unexplained infertility. *J Assist Reprod Genet* 2003;20:377-81.
- Aboulghar MA, Mansour RT, Serour GI, Sattar MA, Amin YM. Intracytoplasmic sperm injection and conventional in vitro fertilization for sibling oocytes in cases of unexplained infertility and borderline semen. *J Assist Reprod Genet* 1996;13:38-42.
- Ruiz A, Remohi J, Minguez Y, Guanes PP, Simon C, Pellicer A. The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. *Fertil Steril* 1997; 68:171-3.
- Bungum L, Bungum M, Humaidan P, Andersen CY. A strategy for treatment of couples with unexplained infertility who failed to conceive after intrauterine insemination. *Reprod Biomed Online* 2004;8:584-9.
- Check JH, Bollendorf A, Summers-Chase D, Horwath D, Hourani W. Conventional oocyte insemination may result in a better pregnancy outcome than intracytoplasmic sperm injection (ICSI) for unexplained infertility. *Clin Exp Obstet Gynecol* 2009;36:150-1.
- Kim HH, Bundorf MK, Behr B, McCallum SW. Use and outcomes of intracytoplasmic sperm injection for non-male factor infertility. *Fertil Steril* 2007;88: 622-8.
- Foong SC, Fleetham JA, O'Keane JA, Scott SG, Tough SC, Greene CA. A prospective randomized trial of conventional in vitro fertilization versus intracytoplasmic sperm injection in unexplained infertility. *J Assist Reprod Genet* 2006;23:137-40.
- Aboulghar MA, Mansour RT, Serour GI, Amin YM, Kamal A. Prospective controlled randomized study of in vitro fertilization versus intracytoplasmic sperm injection in the treatment of tubal factor infertility with normal semen parameters. *Fertil Steril* 1996;66:753-6.
- Moreno C, Ruiz A, Simon C, Pellicer A, Remohi J. Intracytoplasmic sperm injection as a routine indication in low responder patients. *Hum Reprod* 1998; 13:2126-9.
- Luna M, Bigelow C, Duke M, Ruman J, Sandler B, Grunfeld L, Copperman AB. Should ICSI be recommended routinely in patients with four or fewer oocytes retrieved? *J Assist Reprod Genet* 2011;28(10):911-5.
- Roest J, Van Heusden AM, Zeilmaker GH, Verhoeff A. Treatment policy after poor fertilization in the first IVF cycle. *J Assist Reprod Genet* 1998;15:18-21.
- Lipitz S, Rabinovici J, Goldenberg M, Bider D, Dor J, Mashiah S. Complete failure of fertilization in couples with mechanical infertility: implications for subsequent in vitro fertilization cycles. *Fertil Steril* 1994;61:863-6.
- Kinzer DR, Barret B, Powers RD. Prognosis for clinical pregnancy and delivery after total fertilization failure during conventional in vitro fertilization of intracytoplasmic sperm injection. *Fertil Steril* 2008;90:284-8.
- Van der Westerlaken, Helmerhorst F, Dieben S, Naaktgeboren N. Intracytoplasmic sperm injection as treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization. *Fertil Steril* 2005;83:612-7.
- Tucker M, Graham J, Han T, Stillman R, Levy M. Conventional insemination versus intracytoplasmic sperm injection. *Lancet* 2001;358:1645-6.
- Abu-Hassan D, Al-Hasani S. The use of ICSI for all cases of in-vitro conception. *Hum Reprod* 2003;18:893-5.
- Bhattacharya S, Hamilton MP, Shaaban M, Khalaf Y, Seddler M, et al. Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. *Lancet* 2001;357:2075-9.
- Yang D, Shahata MA, al-Bader M, al-Natsha SD, al-Flamerzia M, al-Shawaf T. Intracytoplasmic sperm injection improving embryo quality: comparison of the sibling oocytes of non-male-factor couples. *J Assist Reprod Genet* 1996;13:351-5.
- Staessen C, Camus M, Clasen K, De Vos A, Van Steirteghem A. Conventional in-vitro fertilization versus intracytoplasmic sperm injection in sibling oocytes from couples with tubal infertility and normozoospermic semen. *Hum Reprod* 1999;14:2474-9.
- Bukulmez O, Yarali H, Yucel A, Sari T, Gurgan T. 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* 2000;73: 38-42.
- Poehl M, Hologschwandtner M, Bichler K, Krischker U, Jurgen S, Feichtinger W. IVF-patients with nonmale factor "to ICSI" or "not to ICSI" that is the question? *J Assist Reprod Genet* 2001;18:205-8.
- Thornhill AR, deDie-Smulders CE, Geraedts JP, Harper JC, Harton GL, et al. ESHRE PGD Consortium 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. *Hum Reprod* 2005;20:35-48.
- Intracytoplasmic sperm injection (ICSI) in 2006: evidence and evolution. *Hum Reprod Update* 2007;13:515-26.
- Hwang JL, Lin YH, Tsai YL. In vitro maturation and fertilization of immature oocytes: a comparative study of fertilization techniques. *J Assist Reprod Genet* 2000;17:39-43.
- Nagy ZP, Cecile J, Liu J, Loccufer A, Devroey P, Van Steirteghem A. Pregnancy and birth after intracytoplasmic sperm injection of in vitro matured germinal-vesicle stage oocytes: case report. *Fertil Steril* 1996;65:1047-50.
- Gook DA, Edgar DH. Human oocyte cryopreservation. *Hum Reprod Update* 2007;13:591-605.
- Bonduelle M, Camus M, De Vos A, Staessen C, Tournaye H, et al. Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. *Hum Reprod* 1999;14(Suppl 1):243-64.
- Bowen JR, Gibson FL, Leslie GI, Saunders DM. Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. *Lancet* 1998;351:1529-34.

39. Leslie GI, Gibson FL, McMahon C, Cohen J, Saunders DM, Tennant C. Children conceived using ICSI do not have an increased risk of delayed mental development at 5 years of age. *Hum Reprod* 2003;18:2067–72.
40. Place I, Englert Y. A prospective longitudinal study of the physical, psychomotor, and intellectual development of singleton children up to 5 years who were conceived by intracytoplasmic sperm injection compared with children conceived spontaneously and by in vitro fertilization. *Fertil Steril* 2003;80:1388–97.
41. Ponjaert-Kristoffersen I, Bonduelle M, Barnes J, Nekkebroeck J, Loft A, et al. International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived, and naturally conceived 5-year-old child outcomes: cognitive and motor assessments. *Pediatrics* 2005;115:e283–9.
42. Meschede D, Lemcke B, Exeler JR, De Geyter C, Behre HM, et al. Chromosome abnormalities in 447 couples undergoing intracytoplasmic sperm injection—prevalence, types, sex distribution and reproductive relevance. *Hum Reprod* 1998;13:576–82.
43. Aboulghar H, Aboulghar M, Mansour R, Serour G, Amin Y, Al-Inany H. A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection. *Fertil Steril* 2001;76:249–53.
44. Bonduelle M, Van Assche E, Joris H, Keymolen K, Devroey P, et al. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod* 2002;17:2600–14.
45. Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med* 2002;346:725–30.
46. Bonduelle M, Wennerholm UB, Loft A, Tarlatzis BC, Peters C, et al. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Hum Reprod* 2005;20:413–9.
47. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, Haan EA, Chan A. Reproductive technologies and the risk of birth defects. *N Engl J Med* 2012;366:1803–13.