Reproductive BioMedicine Online (2012) **25**, 603–607

www.sciencedirect.com
www.rbmonline.com

ARTICLE

IVF versus ICSI for the fertilization of in-vitro matured human oocytes

M Walls [a,b,*], S Junk [a], JP Ryan [a], R Hart [a,b]

[a] *Fertility Specialists WA, 25 Queenslea Drive, Claremont, Perth, Western Australia 6010, Australia;* [b] *School of Women's and Infant's Health, University of Western Australia, King Edward Memorial Hospital, 374 Bagot Road, Subiaco, Perth, Western Australia 6008, Australia*

[*] Corresponding author. *E-mail address:* melanie@fertilitywa.com.au (M Walls).

Melanie Walls graduated with a bachelor of biomedical science at the University of Notre Dame in 2007 before beginning her training in embryology in 2008 at the Fertility Specialists WA in Perth where she is currently employed as a qualified embryologist. In 2011 she completed an honours in medical science with the School of Women's and Infants' Health at the University of Western Australia. Melanie has recently embarked on a PhD with her main research interest of in-vitro maturation.

Abstract Traditional dogma suggests that intracytoplasmic sperm injection (ICSI) should be performed to ensure successful oocyte fertilization in an in-vitro maturation (IVM) cycle. This study postulated that there would be no difference in the fertilization rate when ICSI was compared with IVF. This hypothesis was tested in a randomized trial of IVF versus ICSI in IVM. A total of 150 immature oocytes were collected in eight cycles of IVM for patients diagnosed with polycystic ovarian syndrome (PCOS). Patients were primed with minimal FSH before transvaginal oocyte aspiration. Sibling oocytes were inseminated by 50% IVF and 50% ICSI. There was no significant difference in fertilization, useable or total blastocyst development between the two insemination technique groups. Clinical pregnancy results for combined fresh and cryopreserved transfers were identical between the two insemination techniques with a total of two fresh and five cryopreserved IVF-inseminated embryos resulting in three clinical pregnancies (42.9%) and five fresh and two cryopreserved ICSI-derived embryos resulting in three clinical pregnancies (42.9%). This research has shown IVF to be a legitimate fertilization technique for IVM oocytes in PCOS patients and provides a greater awareness of the use of a fertilization method previously not utilized with IVM.

© 2012, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: fertilization, ICSI, IVM, IVF, PCOS, pregnancy

Introduction

In-vitro maturation (IVM) is a novel technique in assisted reproductive technology which was developed as an alternative to traditional IVF due to the adverse outcomes of ovarian hyperstimulation syndrome and the costs associated with the administration of FSH. The treatment also has the potential to overcome other causes of infertility such as male factor, gamete donation and poor response to stimulation, and also has profound benefits for women undergoing oocyte or embryo cryopreservation with an oestrogen-sensitive tumour or with a prothrombotic medical

1472-6483/$ - see front matter © 2012, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.rbmo.2012.08.001

condition. To date, the literature has focused on oocyte maturation and embryo development as affected by culture condition (Goud et al., 1998; Benkhalifa et al., 2009; Ben-Ami et al., 2011; Mikkelsen et al., 2000), stimulation protocols (Cha et al., 2000; Hreinsson et al., 2003; Lin et al., 2003; Liu et al., 2003; Mikkelsen and Lindenberg, 2001) and patient selection (Child et al., 2001; Fridén et al., 2005) with very few studies assessing the method of fertilization techniques (Hwang et al., 2000; Lacham-Kaplan and Trounson, 2008; Söderström-Anttila et al., 2005).

In clinical human IVM cases, the process of fertilization has been performed almost exclusively by intracytoplasmic sperm injection (ICSI). The limited amount of research into the application of traditional IVF as a fertilization technique has shown promising results (Söderström-Anttila et al., 2005). Further research is needed to determine whether IVF is a viable option for IVM patients.

Materials and methods

Patient cohort

A total of eight patients with polycystic ovary syndrome (PCOS) were recruited into the study, resulting in eight fresh and an additional seven cryopreserved embryo transfer cycles between May 2011 and February 2012. The patients underwent a consultation with their clinician to assess eligibility for treatment. Eligibility was assessed as those women exhibiting polycystic ovaries on ultrasound (i.e. $\geq$10 small follicles of 2–8 mm in diameter in at least one ovary) with the male partner having normal semen parameters (World Health Organization, 2010) and at least one of the following: elevated LH ($\geq$10 IU/l), elevated free androgen index ($>$6.0), cyclic disturbances, ranging from oligomenorrhoea to complete amenorrhoea, body mass index $>30\ kg/m^2$ or hirsutism. Once eligibility was established, appropriate consent was obtained and patients were recruited into the study, providing the semen parameters fell within the guidelines for IVF. Ethical approval for the study was received from the Human Research Ethics Committee of Curtin University on 10 March 2011 (approval reference number HR 16/2011).

Treatment regime

On day 2 of the patient's menstrual cycle, the patients had a blood test for circulating hormone concentrations and were considered ready to commence treatment once the following were achieved; oestrogen ($\leq$250 pmol/l), progesterone ($\leq$3.5 nmol/l), FSH ($\leq$10 IU/l), LH ($\leq$10 IU/l) and prolactin ($\leq$500 μU/l). A transvaginal ultrasound scan was performed to determine the number of antral follicles on each ovary. If the patient was determined to be ready, gonadotrophins were administered for 3–5 days subcutaneously (FSH priming). The normal dosage being 150 IU FSH using either Gonal-F (Merck-Serono, Frenchs Forest, NSW, Australia) or Puregon (Schering-Plough, North Ryde, NSW, Australia). On day 6 of the cycle, the patient underwent an additional transvaginal ultrasound scan and these were repeated every 2 days until a follicle approximately 1 cm in diameter was observed, at which time the patient was considered ready for oocyte collection within the following 72 h.

Endometrial preparation

Hormone replacement therapy was administered to prepare the endometrium for implantation following embryo culture. Two days prior to egg collection, patients were administered 3 mg of oestradiol valerate (Progynova; Schering-Plough) orally three times per day. On the day of egg collection, the dose was decreased to 2 mg oestradiol valerate orally three times per day. Twenty-four hours post egg collection, the patient commenced 400 mg progesterone pessaries (Emslies Pharmacy Perth, WA) three times per day or Crinone (Merck-Serono) 90 mg twice a day. The progesterone regime continued until the pregnancy test 15 days post embryo transfer. If the test was positive, the regime continued for 12 weeks of pregnancy; if negative, the regime was ceased.

Oocyte collection

Oocytes were collected from 2–10 mm follicles under transvaginal ultrasound guidance using a 20-gauge double-lumen needle (Cook Medical, Brisbane, Queensland, Australia). Cumulus–oocyte-complexes (COC) were identified and removed from the collection fluid using a sterile glass pipette and washed in G-IVFPlus medium (Vitrolife, Sweden) in a small Petri dish. The COC were then transferred to G-2Plus medium (Vitrolife) supplemented with 10% heat-inactivated maternal serum.

Oocyte maturation and culture

Oocyte maturation culture of cumulus-enclosed germinal-vesicle (GV) oocytes was performed in 20 μl droplets of G-2Plus medium supplemented with 10% maternal serum, 0.1 IU/ml FSH (Puregon) and 0.5 IU/ml human chorionic gonadotrophin (HCG; Pregnyl; Schering-Plough) under sterile mineral oil. The immature eggs were cultured at 37°C in an atmosphere of 6% CO_2, 5% O_2 and 89% N_2 for 24 h prior to insemination.

Insemination via split fertilization of collected oocytes

The semen sample was prepared on the day following oocyte retrieval by placing a portion of the specimen over a discontinuous (40% and 80%) PureSperm (Nidacon, Sweden) gradient and buoyancy density centrifuging for 10 min at 593*g*. The sperm pellet was extracted, washed twice and resuspended in G-IVFPlus medium. After culture for 24 h, 50% of the oocytes were randomly selected to be checked for nuclear maturation and later inseminated by ICSI using the husband's prepared spermztozoa. The remaining 50% of IVM oocytes were inseminated by the addition of approximately 120,000 spermztozoa to the culture dish. All

oocytes were checked for signs of fertilization after 16–18 h. Immature GV and metaphase-I oocytes were disregarded from fertilization results in both groups.

Fertilization check and embryo culture

Approximately 16–18 h post-insemination/injection, the oocytes were checked under the microscope for signs of fertilization. They were deemed to be fertilized by the presence of two pronuclei and two polar bodies. The embryos were transferred to G-1Plus medium for a further 48 h. The embryos were then transferred to 20 μl G2Plus medium and cultured for an additional 48 h. After this time, embryo development was assessed and blastocyst-stage embryos were graded (Dokras et al., 1993). Grade 1 blastocysts had good cellular development of both the inner cell mass (ICM) and trophectoderm (TE), grade 2 blastocysts had average development of the ICM and TE, while grade 3 had poor cellular development. For embryo transfer or vitrification, only grade 1 or 2 embryos were selected.

Embryo transfer and pregnancy test

The embryo selected for transfer was chosen according to the highest morphological grade by an embryologist blinded to the insemination technique from which it was derived. Patients were tested for pregnancy 10 days after embryo transfer by a blood test for serum HCG concentration and the results recorded. A clinical pregnancy was classified by the presence of a fetal sac on ultrasound scan 4 weeks after a positive blood HCG result. Supernumerary grades 1 and 2 blastocysts were cryopreserved by vitrification (Cook Medical) for future treatment.

Data analysis

Univariate analysis of statistical data were performed using chi-squared test with fisher's Exact method. $P < 0.05$ was considered significant. The statistical package SPSS for Windows 10 (SPSS, Chicago, USA) was used for all statistical calculations.

Results

A total of 150 immature oocytes were collected in eight IVM cycles. The mean ± SD oocytes collected per transvaginal oocyte aspiration was 18.75 ± 5.33. There were 72 immature oocytes subsequently inseminated using IVF, of which 57 (79.2%) were found to be mature (metaphase II) at the time of the fertilization check. There were 78 COC denuded for insemination by ICSI and 56 (71.8%) were found to be mature. There was no difference in fertilization percentages between the two insemination technique groups. A total of 34 of the 57 (59.6%) mature oocytes in the IVF group fertilized normally. In contrast, normal fertilization was observed in 38 of the 56 (67.9%) mature oocytes in the ICSI group. The degeneration rate after ICSI insemination was 13.8% with no observed degeneration in the IVF group. No patient recorded a complete failed fertilization in either the IVF or the ICSI group.

There were no significant differences between the IVF and ICSI groups in useable or total blastocyst formation rates (Table 1). For the IVF group, 14 of the 34 (41.2%) normally fertilized embryos went on to form useable blastocysts whereas 18 of the 38 (47.4%) of the ICSI-fertilized embryos were useable and deemed suitable for either transfer or vitrification. A comparison of total blastocyst formation rates, regardless of morphological grade, also demonstrated similar rates of development. For the IVF group, 21 of the 34 (61.8%) normally fertilized embryos

Table 1 Recruited patient outcomes of IVM treatment.

	IVM–IVF	*IVM–ICSI*
Oocytes		
Total	72	78
Per patient	9.0 ± 2.83	9.75 ± 2.55
Oocytes matured		
Total	57/72 (79.2)	56/78 (71.8)
Per patient	7.1 ± 2.23	7.0 ± 1.6
Matured oocytes fertilized		
Total	34/57 (59.6)	38/56 (67.9)
Per patient	4.2 ± 2.05	4.75 ± 1.16
Useable blastocysts formed		
Total	14/34 (41.2)	18/38 (47.4)
Per patient	1.75 ± 1.39	2.25 ± 1.04
Blastocysts formed		
Total	21/34 (61.8)	21/38 (55.3)
Per patient	2.6 ± 1.5	2.63 ± 1.06

Values are *n*, *n*/total (%) or mean ± SD.
There were no statistically significant differences between the two groups.
ICSI = intracytoplasmic sperm injection; IVM = in-vitro maturation.

Table 2 Comparison of embryonic outcomes according to fertilization technique.

	IVF	*ICSI*
Useable blastocysts	14	18
Vitrified blastocysts	12	13
Embryos transferred	7	7
Fresh cycle	2	5
Cryopreserved cycle	5	2
Sacs/fetal heart	3	3
Cumulative implantation rate (%)	42.86	42.86

Values are *n* unless otherwise stated.
ICSI = intracytoplasmic sperm injection.

went on to form blastocysts compared with 21 of the 38 (55.3%) ICSI-fertilized embryos.

Successful embryo development to the blastocyst stage resulted in 14 IVF embryos and 18 ICSI-derived embryos being transferred or cryopreserved (**Table 2**). Overall, six of the eight PCOS patients recorded a positive biochemical pregnancy and all six established viable ongoing pregnancies following the transfer of fresh or cryopreserved embryos. A cumulative implantation rate of 42.9% was observed after seven IVF-derived single-embryo transfers resulted in three ongoing pregnancies (two fresh cycles and five cryopreserved cycles). An identical cumulative implantation rate (42.9%) was observed for ICSI-derived embryos: three ongoing pregnancies from seven single-embryo transfers (five fresh cycles and two cryopreserved cycles). There were no reported cases of early miscarriage.

Discussion

IVM has the potential to treat a wide range of infertility conditions aside from PCOS, such as male factor infertility and poor response to traditional stimulation treatments, and is an option for fertility preservation prior to the treatment for hormone-sensitive cancers. As far as is known, this is the first study of its kind to assess embryo culture to the blastocyst stage of IVM oocytes fertilized by IVF allowing for preferential use of single-embryo transfers. The use of sibling oocytes to compare outcomes in this study meant that fertilization rates were more easily compared between the IVF and ICSI groups. The use of sibling oocytes may account for the improved fertilization outcome of the IVF group when compared with that of the only other human clinical study assessing fertilization techniques (Söderström-Anttila et al., 2005), which found an overall IVF fertilization rate of 37.7% and a PCOS-specific fertilization rate of 43.8%, which are somewhat lower than the current rate of 59.6%, and ICSI fertilization rates of 69.3% overall and 78.4% PCOS specific, which are similar to the current rate of 67.9%.

It is important to address a potential flaw in directly comparing the fertilization outcomes from the two test groups, as the time points for assessing maturity differ significantly. By assessing the maturity of the oocytes for the ICSI group prior to insemination, there is a potential selection bias by only inseminating those known to be mature. Those inseminated by IVF are assessed for both maturity and fertilization at the same time and therefore include any oocytes which, had they been checked at the same time as the ICSI group, could potentially have been immature and have subsequently undergone spontaneous maturation overnight. Studies have shown those oocytes that take longer to undergo polar body extrusion have a decreased ability to fertilize, reduced potential for normal embryo development and an increased risk of aneuploidy (Emery et al., 2005).

Higher fertilization rates have previously been reported for ICSI-inseminated patients (72%) compared with IVF inseminations (45%) for conventional non-IVM treatment of PCOS patients (Hwang et al., 2005). This fertilization percentage was calculated including cases of failed fertilization. However, even after the removal of such cases of complete failure of fertilization, a reduced rate of fertilization of 53% for IVF was observed compared with 72% for ICSI (Hwang et al., 2005). The decrease in fertilization capacity and an increase in the proportion of cases of failed fertilization has been attributed to potentially poor oocyte quality as a result of long-term abnormal hormonal milieu seen in PCOS patients (Hwang et al., 2005). There were no cases of failed fertilization, which may provide a partial explanation for the improved IVF fertilization outcome compared with ICSI. The occurrence of failed fertilization is reported as more evident in IVF than in ICSI, with percentages ranging from 1.5% versus 2.1% (Ola et al., 2001), 11% versus 0% (Ruiz et al., 1997) and as high as 15% versus 1.17% (Hwang et al., 2005). Indeed, the risk of a complete failure of fertilization was assumed to be higher in IVM cases due to longer culture periods resulting in zona pellucida hardening (Nagy et al., 1996). Rates of failed fertilization have also been shown to be higher in PCOS patients (18%) compared with non-PCOS patients (5%) (Kodama et al., 1995). Rates of oocyte degeneration are also important when evaluating the efficiencies of the two insemination methods. The two insemination techniques are radically different in the way they are performed, with the ICSI procedure physically disrupting the ooplasm and oolemma to allow sperm penetration, and therefore the two techniques cannot be directly compared for degeneration rates. However, it is a factor which must be considered when evaluating which technique to use in treatment. If these oocytes were inseminated using IVF, their risk of degeneration would have been greatly decreased, although degeneration following ICSI may reflect oocytes of poor quality which may not have fertilized under any circumstances.

Development to the blastocyst stage is rarely reported in a clinical human setting for embryos derived from IVM. The similarities in development between the two groups in the present study indicate that IVM oocytes are developmentally competent to progress to the blastocyst stage, having achieved both nuclear and cytoplasmic maturation. The results also indicate that development to the blastocyst stage is not only achievable but consistent with results from conventional treatment (Van Landuyt et al., 2005). The blastocyst development rates of 41.2% useable and 61.8% total for the IVF-inseminated group is consistent with those achieved by Griffiths et al. (2000), who demonstrated a 50% development for the IVF group in a conventional stimulated cycle. The current ICSI results (47.4% and 55.3% for useable and total blastocyst development, respectively), however, are superior to those reported by Griffiths et al. (2000) of 20%, but are similar to that achieved (50.4%) under similar culture conditions and maturation timing (Son et al., 2005). Blastocyst development in conventional stimulation cycles has proven to be an effective determinant of embryo quality to assist with higher rates of implantation and ongoing pregnancy, while allowing for the wider use of single-embryo transfer and lowering the rate of multiple births worldwide (Gardner et al., 1998).

The outcomes from this study suggest that IVF is a valid fertilization technique for the insemination of IVM oocytes from PCOS patients with normal semen parameters. The lack of a significant difference in fertilization rates between the ICSI and IVF groups indicates that IVF can be used to achieve acceptable fertilization rates. The similarity in

embryo development rates at the cleavage, useable and total blastocyst development stages shows that oocytes inseminated by IVF are just as capable of producing viable embryos as ICSI-inseminated oocytes In terms of embryo yield, this study has shown that patients are just as likely to achieve a blastocyst-stage transfer if they have their eggs fertilized by IVF as they are if they are fertilized by ICSI. With the improvements in IVM culture conditions and blastocyst development rates in recent years, there is a need for further research into the utilization of IVF in conjunction with IVM treatment and its potential for creating more viable embryos with improved implantation and pregnancy potential while being a more cost-effective and less invasive form of treatment.

Acknowledgements

The authors would like to thank the staff who assisted with this study at the Fertility Specialists WA and the University of Western Australia, School of Women's and Infant's Health.

References

Ben-Ami, I., Komsky, A., Bern, O., Kasterstein, E., Komarovsky, D., Ron-El, R., 2011. In vitro maturation of human germinal vesicle-stage oocytes: role of epidermal growth factor-like growth factors in the culture medium. Hum. Reprod. 26, 76–81.

Benkhalifa, M., Demirol, A., Menezo, Y., Balashova, E., Abduljalil, A.K., Abbas, S., Giakoumakis, I., Gurgan, T., 2009. Natural cycle IVF and oocyte in-vitro maturation in polycystic ovary syndrome: a collaborative prospective study. Reprod. Biomed. Online 18, 29–36.

Cha, K.Y., Han, S.Y., Chung, H.M., Choi, D.H., Lim, J.M., Lee, W.S., Ko, J.J., Yoon, T.K., 2000. Pregnancies and deliveries after in vitro maturation culture followed by in vitro fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. Fertil. Steril. 73, 978–983.

Child, T.J., Abdul-Jalil, A.K., Gulekli, B., Lin Tan, S., 2001. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. Fertil. Steril. 76, 936–942.

Dokras, A., Sargent, I.L., Barlow, D.H., 1993. Fertilization and early embryology: human blastocyst grading: an indicator of developmental potential? Hum. Reprod. 8, 2119–2127.

Emery, B.R., Wilcox, A.L., Aoki, V.W., Peterson, C.M., Carrell, D.T., 2005. In vitro oocyte maturation and subsequent delayed fertilization is associated with increased embryo aneuploidy. Fertil. Steril. 84, 1027–1029.

Fridén, B., Hreinsson, J., Hovatta, O., 2005. Birth of a healthy infant after in vitro oocyte maturation and ICSI in a woman with diminished ovarian response: case report. Hum. Reprod. 20, 2556–2558.

Gardner, D.K., Schoolcraft, W.B., Wagley, L., Schlenker, T., Stevens, J., Hesla, J., 1998. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. Hum. Reprod. 13, 3434–3440.

Goud, P.T., Goud, A.P., Qian, C., Laverge, H., Van Der Elst, J., De Sutter, P., Dhont, M., 1998. In-vitro maturation of human germinal vesicle stage oocytes: role of cumulus cells and epidermal growth factor in the culture medium. Hum. Reprod. 13, 1638–1644.

Griffiths, T.A., Murdoch, A.P., Herbert, M., 2000. Embryonic development in vitro is compromised by the ICSI procedure. Hum. Reprod. 15, 1592–1596.

Hreinsson, J., Rosenlund, B., Fridén, B., Levkov, L., Ek, I., Suikkari, A.M., Hovatta, O., Fridström, M., 2003. Recombinant LH is equally effective as recombinant hCG in promoting oocyte maturation in a clinical in-vitro maturation programme: a randomized study. Hum. Reprod. 18, 2131–2136.

Hwang, J.-L., Lin, Y.-H., Tsai, Y.-L., 2000. In vitro maturation and fertilization of immature oocytes: a comparative study of fertilization techniques. J. Assist. Reprod. Genet. 17, 39–43.

Hwang, J.-L., Seow, K.-M., Lin, Y.-H., Hsieh, B.-C., Huang, L.-W., Chen, H.-J., Huang, S.-C., Chen, C.-Y., Chen, P.-H., Tzeng, C.-R., 2005. IVF versus ICSI in sibling oocytes from patients with polycystic ovarian syndrome: a randomized controlled trial. Hum. Reprod. 20, 1261–1265.

Kodama, H., Fukuda, J., Karube, H., Matsui, T., Shimizu, Y., Tanaka, T., 1995. Endocrinology: high incidence of embryo transfer cancellations in patients with polycystic ovarian syndrome. Hum. Reprod. 10, 1962.

Lacham-Kaplan, O., Trounson, A., 2008. Reduced developmental competence of immature, in-vitro matured and postovulatory aged mouse oocytes following IVF and ICSI. Reprod. Biol. Endocrinol. 6, 58.

Lin, Y.H., Hwang, J.L., Huang, L.W., Mu, S.C., Seow, K.M., Chung, J., Hsieh, B.C., Huang, S.C., Chen, C.Y., Chen, P.H., 2003. Combination of FSH priming and hCG priming for in-vitro maturation of human oocytes. Hum. Reprod. 18, 1632–1636.

Liu, J., Lu, G., Qian, Y., Mao, Y., Ding, W., 2003. Pregnancies and births achieved from in vitro matured oocytes retrieved from poor responders undergoing stimulation in in vitro fertilization cycles. Fertil. Steril. 80, 447–449.

Mikkelsen, A., Lindenberg, S., 2001. Benefit of FSH priming of women with PCOS to the in vitro maturation procedure and the outcome: a randomized prospective study. Reproduction 122, 587–592.

Mikkelsen, A.L., Smith, S., Lindenberg, S., 2000. Impact of oestradiol and inhibin A concentrations on pregnancy rate in in-vitro oocyte maturation. Hum. Reprod. 15, 1685–1690.

Nagy, Z.P., Cecile, J., Liu, J., Loccufier, A., Devroey, P., Van Steirteghem, A., 1996. Pregnancy and birth after intracytoplasmic sperm injection of in vitro matured germinal-vesicle stage oocytes: case report. Fertil. Steril. 65, 1047.

Ola, B., Afnan, M., Sharif, K., Papaioannou, S., Hammadieh, N., Lr, B., 2001. Should ICSI be the treatment of choice for all cases of in-vitro conception? Hum. Reprod. 16, 2485.

Ruiz, A., Remohí, J., Minguez, Y., Guanes, P.P., Simón, C., Pellicer, A., 1997. The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. Fertil. Steril. 68, 171–173.

Söderström-Anttila, V., Mäkinen, S., Tuuri, T., Suikkari, A.-M., 2005. Favourable pregnancy results with insemination of in vitro matured oocytes from unstimulated patients. Hum. Reprod. 20, 1534–1540.

Son, W.-Y., Lee, S.-Y., Lim, J.-H., 2005. Fertilization, cleavage and blastocyst development according to the maturation timing of oocytes in in vitro maturation cycles. Hum. Reprod. 20, 3204–3207.

Van Landuyt, L., De Vos, A., Joris, H., Verheyen, G., Devroey, P., Van Steirteghem, A., 2005. Blastocyst formation in in vitro fertilization versus intracytoplasmic sperm injection cycles: influence of the fertilization procedure. Fertil. Steril. 83, 1397–1403.

World Health Organization, 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen. WHO Press, Geneva.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 29 May 2012; refereed 25 July 2012; accepted 16 August 2012.