Reproductive BioMedicine Online (2010) **20**, 634–638

www.sciencedirect.com
www.rbmonline.com

ARTICLE

In vitro maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation

K Oktay [a,*,1], E Buyuk [b,1], KA Rodriguez-Wallberg [a,c], G Sahin [a]

[a] *Institute for Fertility Preservation and Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, New York Medical College and Westchester Medical Center, Munger Pavilion, Rm-617, Valhalla, NY 10595, USA;* [b] *Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Albert Einstein College of Medicine and Montefiore, Institute for Reproductive Medicine and Health, 141 S Central Avenue, Ste 201, Hartsdale, NY 10530, USA;* [c] *Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden*

[*] Corresponding author. *E-mail address:* koktay@fertilitypreservation.org (K Oktay). [1] Professor Oktay and Dr Buyuk contributed equally to this manuscript.

Kutluk Oktay is one of the world's leading experts in fertility preservation. He developed and performed the world's first ovarian transplantation procedures as well as pioneering innovative ovarian stimulation protocols for embryo and oocyte freezing for breast and endometrial cancer patients. He founded and was elected as the first President of the Fertility Preservation Special Interest Group of the American Society for Reproductive Medicine.

Abstract This study tested in-vitro maturation (IVM) as a complementary strategy to improve the mature oocyte yield of breast cancer patients undergoing ovarian stimulation for fertility preservation. Secondary analysis of prospectively collected data is performed for 32 breast cancer patients undergoing oocyte or embryo cryopreservation before chemotherapy. Total number of oocytes and/or embryos cryopreserved following IVM is compared with the total number cryopreserved before IVM. Overall, 464 oocytes were retrieved, of which 274 were mature. Following IVM, the number of total mature oocytes increased to 399 (45% increase in mature oocyte yield, $P < 0.0001$). Fertilization rate after IVM was statistically significantly higher than the fertilization of already mature oocytes at retrieval (86% versus 73%, respectively, $P < 0.05$). The total number of oocytes and embryos frozen before IVM was 207 (45% of all oocytes retrieved). This number increased to 320 (69% of all oocytes retrieved) following IVM ($P < 0.0001$). IVM is a useful strategy to improve the mature oocyte yield of fertility preservation cycles. Immature oocytes retrieved during oocyte/embryo cryopreservation cycles should not be discarded to improve the future potential of fertility.

© 2010, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: cryopreservation, embryo, fertility preservation, in-vitro maturation, oocyte

1472-6483/$ - see front matter © 2010, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.rbmo.2010.01.012

Introduction

Preservation of fertility has become a major concern for patients being treated with chemotherapy or pelvic radiotherapy for cancer or other conditions because of the detrimental effects of such treatments on gonadal function (Oktay and Sonmezer, 2008). These patients are primarily offered ovarian stimulation for oocyte or embryo cryopreservation if they have enough time before starting their gonadotoxic treatment. If there is insufficient time for ovarian stimulation, experimental protocols of ovarian tissue cryopreservation and/or germinal vesical stage (GV) oocyte retrieval without ovarian stimulation combined with in-vitro maturation (IVM) are used (Chian et al., 2004; Oktay et al., 2008). For patients undergoing ovarian stimulation, maximizing the number of oocytes or embryos cryopreserved is of utmost importance since these patients may not have a chance for a second IVF cycle before initiating their treatment.

In IVF cycles, 12–20% of the retrieved oocytes are usually immature (Cha and Chian, 1998; De Vos et al., 1999; Van Steirteghem et al., 1993). While immature oocytes are generally discarded in standard IVF cycles, IVM may be important to maximize the number of gametes cryopreserved before sterilizing chemotherapy.

This study was conducted to determine if IVM can increase the yield of mature oocytes and embryos during ovarian stimulation/IVF cycles performed for fertility preservation in breast cancer patients.

Materials and methods

Patients

This is a secondary analysis of prospectively collected data from patients undergoing oocyte or embryo cryopreservation before chemotherapy. Adult patients who had a histologically confirmed breast cancer diagnosis, had both ovaries, regular menstrual cycles and normal basal (day 2 or 3) FSH, LH and oestradiol were included.

In-vitro fertilization protocol

Ovarian stimulation protocol with aromatase inhibitors were approved by the institutional review board and a written informed consent was obtained from each patient. Ovarian stimulation protocols for breast cancer patients were as follows: letrozole 5 mg per day (Femara, Novartis, East Hanover, USA) or anastrozole (Arimidex, AstraZeneca, Wilmington, DE, USA) was administered starting on day 2 of the menstrual cycle. Aromatase inhibitors were continued until the day of human chorionic gonadotrophin (hCG). In anastrozole cycles, initially a dose of 2 mg/day was used. The dose was increased subsequently up to 10 mg/day as needed in an effort to keep oestradiol concentrations below 500 pg/ml. In patients receiving 6 mg/day or more anastrozole, the dose was divided between morning and bedtime. Aromatase inhibitor treatment was followed 2 days later (cycle day 4) by the daily administration of recombinant FSH 150–300 IU (Gonal-F; Serono, Rockville; or Follistim; Organon, West Orange, USA) based on age. A gonadotrophin-releasing hormone antagonist (Ganirelix, 250 μg per day; Organon) was administered when serum oestradiol concentration exceeded 300 pg/ml or the lead follicle size exceeded 13 mm diameter. hCG was administered when the two leading follicles reached 19–20 mm in diameter. Transvaginal oocyte retrieval was performed 36 h later. The retrieved mature oocytes were fertilized by intracytoplasmic sperm injection. To prevent a rebound increase in oestradiol, letrozole treatment was restarted after oocyte retrieval until concentrations fell below 50 pg/ml.

Hormone analysis and cycle monitoring

FSH and LH were measured using a solid-phase chemiluminescent immunometric assay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA). The FSH assay has a sensitivity of 0.1 mIU/ml and the LH assay has a sensitivity of 0.05 mIU/ml. Oestradiol was quantified using in-house radioimmunoassay (Direct ^{125}I; Pantex, Santa Monica, CA, USA). The assay has a minimum sensitivity of 10 pg/ml, an intra-assay coefficient of variation of 4.2–16% and inter-assay coefficient of variation of 7.3–15.5%.

In-vitro maturation and cryopreservation

The cumulus was partially removed immediately after retrieval. Immature oocytes, at GV (PI) stage, were placed in the IVM medium for 24 h. IVM medium was based on sequential IVF medium, (G2; Vitrolife, Englewood, CO, USA) supplemented with 75 mIU/ml FSH (Organon), 10 ng/ml epidermal growth factor (Sigma–Aldrich, St. Louis, MO, USA), and 0.5 mg/l insulin, transferrin, selenium (Sigma–Aldrich). Intracytoplasmic sperm injection (ICSI) was performed on all oocytes matured *in vitro* in embryo cryopreservation cycles. All embryos were frozen at the pronuclear stage. In oocyte cryopreservation cycles, a slow freezing technique was used with propanediol as the cryoprotectant (Fabbri et al., 2000).

Statistical analysis

Patients' demographic characteristics, hormonal data and IVF outcomes were presented as mean ± SEM. Spearman's rank correlation test was used for correlation between groups; Pearson chi-squared test was used to compare proportions; $P < 0.05$ was considered statistically significant. STATA statistical program (STATA, USA) was used for statistical analysis.

Results

Thirty-two breast cancer patients met the inclusion criteria. All patients were in their first IVF cycle and each contributed one cycle to this study. No cycle cancellation occurred. The clinical and cycle characteristics of this cohort are presented in **Table 1**. The mean diameter of the punctured follicles ranged from 11–25 mm. Antral follicle counts (AFC) strongly correlated with the number of immature oocytes retrieved ($r = 0.73$, $P < 0.0001$) (**Figure 1**). In 25 cycles, only embryos were frozen. Oocytes

Table 1 Baseline characteristics of the patients included in the study.

Parameter	*Value*
Number of patients (cycles)	32
Age (years)	34.7 ± 0.7
Day-2 FSH (IU/l)	6.4 ± 0.6
Day-2 LH (IU/l)	4 ± 0.5
Day-2 oestradiol (pg/ml)	37.7 ± 3.2
Day-2 antral follicle count	15.9 ± 1.6
Length of stimulation (days)	10.2 ± 0.3 (range 7—15)
Total gonadotrophin dose (IU)	1987 ± 127.5
Oestradiol on hCG day (pg/ml)	502.8 ± 77.4

Values are mean ± SEM, unless otherwise stated.

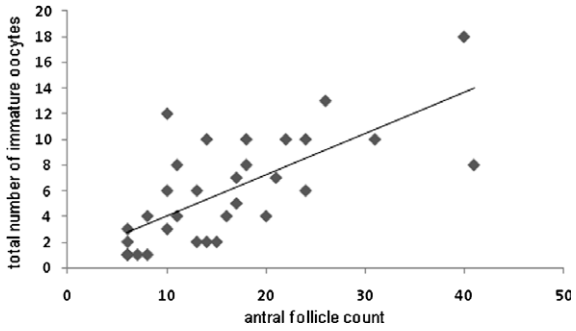

Figure 1 Relationship between antral follicle count and total number of immature oocytes at retrieval (Spearman's *rho* = 0.73, $P < 0.0001$).

were frozen in seven cycles, in three of which embryos were also frozen. The decision to freeze embryos, oocytes or both was determined according to the patients' social circumstances and the number of oocytes recovered.

Complete failure of IVM occurred in two cycles (6.3%). The outcomes of IVM and IVF are presented in **Figure 2**. Of the 464 oocytes that were retrieved, 274 were mature, 174 were at GV or M1 stage, and 16 were degenerate. Of the 174 oocytes placed in IVM media, 125 extruded their first polar bodies, increasing the total number of mature oocytes to 399. This translated into a 45% increase in mature oocyte yield per patient (from 8.6 to 12.5, $P < 0.0001$, 95% CI 3.1—6.0). Of the 274 oocytes that were mature at retrieval, 249 underwent ICSI and of these, 182 (73%) fertilized. Of the 125 oocytes matured by IVM, 87 underwent ICSI and of these, 75 (86%) fertilized. Interestingly, the fertilization rate after IVM was statistically significantly higher than fertilization of already mature oocytes at retrieval ($P < 0.05$, 95% CI 1.1—4.9). The total number of mature oocytes and embryos frozen before IVM was 207 (45% of all oocytes retrieved). This number increased to 320 (69%) following IVM and this translated into a 54% increase in total number of oocytes/embryos cryopreserved per patient (from 6.5 to 10, $P < 0.0001$, 95% CI 1.4—2.4).

Discussion

This study has demonstrated that IVM is a useful strategy to improve the mature oocyte yield for cancer patients undergoing fertility preservation by embryo or oocyte cryopreservation. The number of MII oocytes was increased by 45% allowing a mean of four additional oocytes or embryos to be frozen per cycle. Interestingly, IVM oocytes had higher fertilization competency than oocytes matured *in vivo*. As previously reported with unstimulated cycles (Tan et al., 2002), AFC positively correlated with the number of immature oocytes retrieved.

The proportion of immature oocytes was higher than that reported previously (Cha and Chian, 1998; De Vos et al., 1999; Kim et al., 2000; Van Steirteghem et al., 1993). A possible explanation for this finding is overzealous puncture of small follicles in cancer patients attempting fertility preservation as well as the use of aromatase inhibitors in every patient. The use of letrozole in combination with gonadotrophins is associated with a significantly higher number of follicles >8 mm and >14 mm on day of hCG (Healey et al., 2003; Mitwally and Casper, 2002; Oktay et al., 2006).

This study's IVM rate of 72% was also higher than those reported in prior publications with stimulated cycles that ranged 26.7—45.1% (De Vos et al., 1999; Strassburger et al., 2004; Vanhoutte et al., 2005). However, Kim and co-workers achieved a maturation rate of 66.7% in 168 GV oocytes retrieved after ovarian stimulation with long gonadotrophin-releasing hormone agonist protocol but normal fertilization occurred in only 51.8% (86% in the present study) (Kim et al., 2000). Moreover, Chian et al. (2000) obtained an IVM rate of 84.3% after hCG priming in unstimulated patients with polycystic ovary syndrome (PCOS). High IVM and fertilization rates in the present study can also be ascribed to the fact that the patients were not infertile and had normal ovarian reserve as determined by FSH, oestradiol and AFC (Child et al., 2002a,b; Mikkelsen and Lindenberg, 2001; Tan et al., 2002).

It has been shown that IVM is associated with qualitative changes in the oocyte, including hardening of the zona pellucida (Schroeder et al., 1990; Zhang et al., 1991). Hence, high fertilization rates in the present study may also be attributed to the fact that all patients underwent ICSI and not standard insemination.

Evidence from experiments on mouse oocytes and embryos indicate that aromatase inhibitors do not impair oocyte maturation and embryo development. When mouse pre-antral follicles are cultured in the presence of anastrozole, GV breakdown and polar body formation in response to

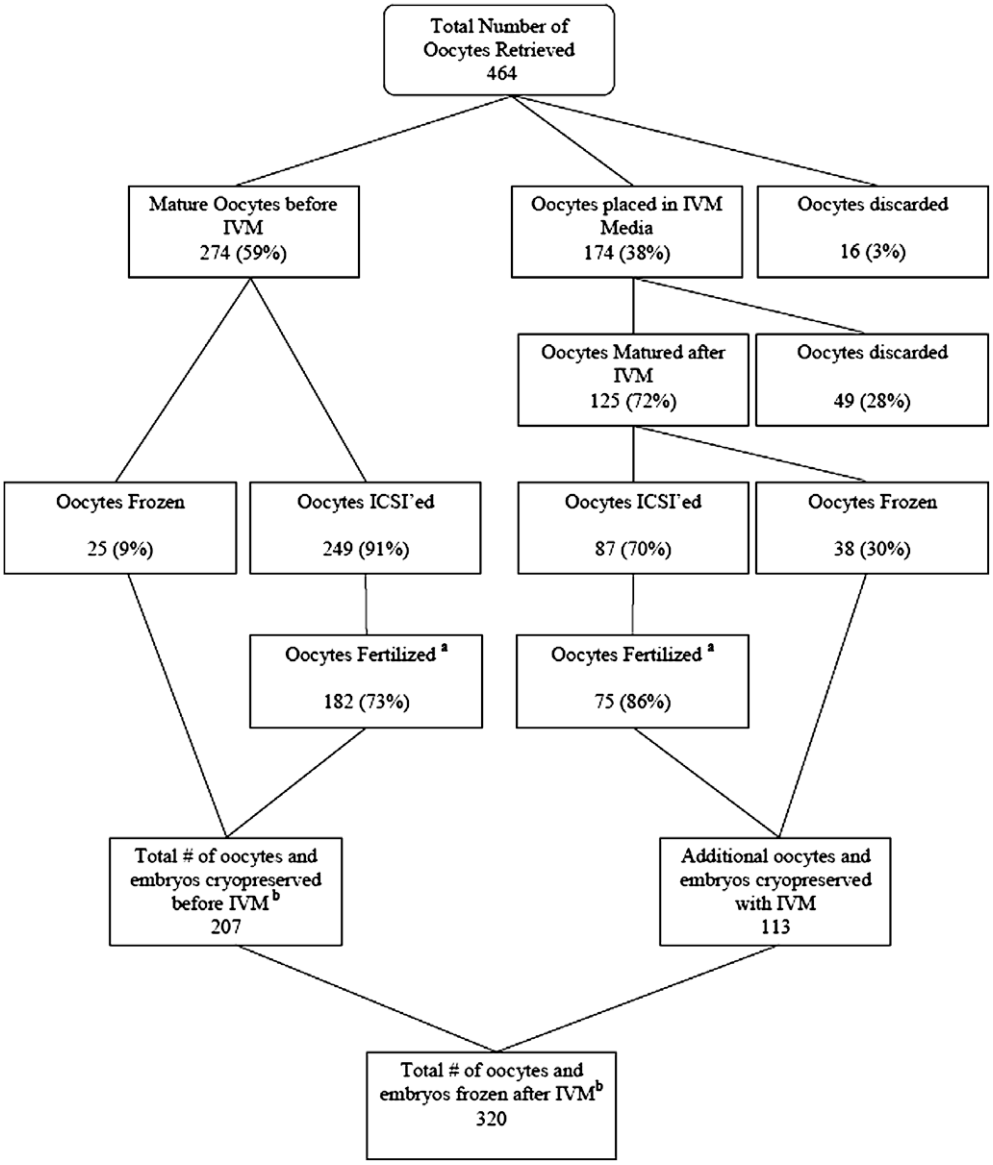

Figure 2 Outcome of all oocytes retrieved. [a]$P < 0.05$, 95% CI: 1.1—4.9 (fertilization rate of mature oocytes at retrieval versus fertilization rate of in-vitro matured oocytes); [b]$P < 0.0001$, 95% CI: 1.4—2.4 (total number of frozen cryopreserved oocytes and embryos before in-vitro maturation versus total number of cryopreserved oocytes and embryos after in-vitro maturation).

hCG are enhanced in a dose-dependent fashion. In that model, maturation rates as high as 90% are encountered in an extremely low oestrogenic environment together with local androgen increase. In the same study, even though a lower fraction of oocytes were fertilized in the presence of anastrozole, a similar number of cleavage stage embryos was obtained (Hu et al., 2002). In another study, in-vivo embryo development was at a more advanced stage when stimulated by anastrozole compared with FSH (Karaer et al., 2005).

Because embryo cryopreservation was performed at the pronuclear stage, embryo development and implantation rates after IVM could not be investigated. Moreover, since this cohort includes only breast cancer patients who are on 5-year tamoxifen treatment following chemotherapy, it will take years for these patients to be cleared for a pregnancy and have their embryos transferred. As a result, the pregnancy outcomes cannot be reported yet. Nevertheless, extrapolating from the current level of success with IVM of oocytes from unstimulated cycles (Holzer et al., 2007) and clinical pregnancy rates of 26—40% with IVM of oocytes from PCOS patients (Child et al., 2002a,b; Zhao et al., 2009), the increase in the number of frozen gametes as a result of IVM of GV oocytes from cancer patients may translate into higher pregnancy rates in the future. It is concluded that immature oocytes obtained during cryopreservation cycles should not be discarded, but should be subjected to IVM to potentially improve the success of fertility preservation.

Acknowledgements

Professor Oktay is supported by a National Institutes of Health Grant (HD53112). Dr Rodriguez-Wallberg is supported

by research grants from The Swedish Society of Medical Research and The Swedish Society of Medicine.

References

Cha, K.Y., Chian, R.C., 1998. Maturation in vitro of immature human oocytes for clinical use. Hum. Reprod. Update 4, 103–120.

Chian, R.C., Buckett, W.M., Tan, S.L., 2004. In-vitro maturation of human oocytes. Reprod. Biomed. Online 8, 148–166.

Chian, R.C., Buckett, W.M., Tulandi, T., Tan, S.L., 2000. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. Hum. Reprod. 15, 165–170.

Child, T.J., Phillips, S.J., Abdul-Jalil, A.K., Gulekli, B., Tan, S.L., 2002b. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. Obstet. Gynecol. 100, 665–670.

Child, T.J., Sylvestre, C., Pirwany, I., Tan, S.L., 2002a. Basal serum levels of FSH and estradiol in ovulatory and anovulatory women undergoing treatment by in-vitro maturation of immature oocytes. Hum. Reprod. 17, 1997–2002.

De Vos, A., Van de Velde, H., Joris, H., Van Steirteghem, A., 1999. In-vitro matured metaphase-I oocytes have a lower fertilization rate but similar embryo quality as mature metaphase-II oocytes after intracytoplasmic sperm injection. Hum. Reprod. 14, 1859–1863.

Fabbri, R., Porcu, E., Marsella, T., et al., 2000. Technical aspects of oocyte cryopreservation. Mol. Cell. Endocrinol. 169, 39–42.

Healey, S., Tan, S.L., Tulandi, T., Biljan, M.M., 2003. Effects of letrozole on superovulation with gonadotropins in women undergoing intrauterine insemination. Fertil. Steril. 80, 1325–1329.

Holzer, H., Scharf, E., Chian, R.C., Demirtas, E., Buckett, W., Tan, S.L., 2007. In vitro maturation of oocytes collected from unstimulated ovaries for oocyte donation. Fertil. Steril. 88, 62–67.

Hu, Y., Cortvrindt, R., Smitz, J., 2002. Effects of aromatase inhibition on in vitro follicle and oocyte development analyzed by early preantral mouse follicle culture. Mol. Reprod. Dev. 61, 549–559.

Karaer, O., Vatansever, H.S., Oruc, S., Ozbilgin, K., Cilaker, S., Koyuncu, M.F., 2005. The aromatase inhibitor anastrozole is associated with favorable embryo development and implantation markers in mice ovarian stimulation cycles. Fertil. Steril. 83, 1797–1806.

Kim, B.K., Lee, S.C., Kim, K.J., Han, C.H., Kim, J.H., 2000. In vitro maturation, fertilization, and development of human germinal vesicle oocytes collected from stimulated cycles. Fertil. Steril. 74, 1153–1158.

Mikkelsen, A.L., 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.

Mitwally, M.F., Casper, R.F., 2002. Aromatase inhibition for ovarian stimulation: future avenues for infertility management. Curr. Opin. Obstet. Gynecol. 14, 255–263.

Oktay, K., Sonmezer, M., 2008. Chemotherapy and amenorrhea: risks and treatment options. Curr. Opin. Obstet. Gynecol. 20, 408–415.

Oktay, K., Demirtas, E., Son, W.Y., Lostritto, K., Chian, R.C., Tan, S.L., 2008. In vitro maturation of germinal vesicle oocytes recovered after premature luteinizing hormone surge: description of a novel approach to fertility preservation. Fertil. Steril. 89, e19–e22.

Oktay, K., Hourvitz, A., Sahin, G., et al., 2006. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J. Clin. Endocrinol. Metab. 91, 3885–3890.

Schroeder, A.C., Schultz, R.M., Kopf, G.S., Taylor, F.R., Becker, R.B., Eppig, J.J., 1990. Fetuin inhibits zona pellucida hardening and conversion of ZP2 to ZP2f during spontaneous mouse oocyte maturation in vitro in the absence of serum. Biol. Reprod. 43, 891–897.

Strassburger, D., Friedler, S., Raziel, A., Kasterstein, E., Schachter, M., Ron-El, R., 2004. The outcome of ICSI of immature MI oocytes and rescued in vitro matured MII oocytes. Hum. Reprod. 19, 1587–1590.

Tan, S.L., Child, T.J., Gulekli, B., 2002. In vitro maturation and fertilization of oocytes from unstimulated ovaries: predicting the number of immature oocytes retrieved by early follicular phase ultrasonography. Am. J. Obstet. Gynecol. 186, 684–689.

Vanhoutte, L., De Sutter, P., Van der Elst, J., Dhont, M., 2005. Clinical benefit of metaphase I oocytes. Reprod. Biol. Endocrinol. 3, 71.

Van Steirteghem, A.C., Liu, J., Joris, H., et al., 1993. Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. Hum. Reprod. 8, 1055–1060.

Zhang, X., Rutledge, J., Armstrong, D.T., 1991. Studies on zona hardening in rat oocytes that are matured in vitro in a serum-free medium. Mol. Reprod. Dev. 28, 292–296.

Zhao, J.Z., Zhou, W., Zhang, W., Ge, H.S., Huang, X.F., Lin, J.J., 2009. In vitro maturation and fertilization of oocytes from unstimulated ovaries in infertile women with polycystic ovary syndrome. Fertil. Steril. 91, 2568–2571.

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

Received 4 August 2009; refereed 18 September 2009; accepted 10 December 2009.