



www.sciencedirect.com
www.rbmonline.com



ARTICLE

The effects of letrozole on ovarian stimulation for fertility preservation in cancer-affected women

Miguel A Checa Vizcaíno ^{a,b,*}, Anna Robles Corchado ^a, Margalida E Sastre I Cuadri ^a, Mireia Gonzalez Comadran ^a, Mario Brassesco ^b, Ramón Carreras ^a


^a Department of Obstetrics and Gynaecology, Hospital del Mar, Universitat Autònoma de Barcelona, Barcelona, Spain;

^b Centro de Infertilidad y Reproducción Humana, Barcelona, Spain

* Corresponding author. E-mail address: macheca@parcdesalutmar.cat (MA Checa Vizcaíno).



Miguel A Checa Vizcaíno obtained his Bachelor of Medicine at the Universitat Autònoma de Barcelona (UAB) in 1994, specialized in gynaecology and obstetrics between 1996 and 2000 and obtained his Doctor of Medicine and Surgery in 2003. Since 2009 he has been Head of Gynaecological Endocrinology and Human Reproduction at Hospital del Mar. He is Associate Professor at UAB, director of various doctorate courses in Gynaecology and director of the International Master in Human Reproduction at UAB. He is currently the co-ordinator of the Gynaecological Endocrinology interest group within the Spanish Society of Fertility (Sociedad Española de Fertilidad).

Abstract Survival rates for fertile women with cancer have increased significantly, lending importance to considering the possibility of motherhood after cancer. This study was a retrospective analysis of a prospective database comparing two groups of patients who underwent fertility preservation after being diagnosed with either breast cancer or a non-hormone-dependent cancer between 2009 and 2011. Nineteen oncology patients were included in the study. The objective was to assess the efficacy of ovarian stimulation with aromatase inhibitors versus a standard antagonist protocol. This study sought to quantify oestradiol concentrations in patients receiving letrozole and to determine the length of time between diagnosis of malignancy and onset of fertility preservation. Number of mature oocytes retrieved in the non-hormone-dependent cancer group was comparable to that in the breast cancer group (15.4 ± 8.19 versus 16.3 ± 7.31). Oestradiol concentrations were higher for patients with non-hormone-dependent cancer (1666.4 ± 739.42 pg/ml versus 829 ± 551.11 pg/ml, $P = 0.006$). There were no differences between the groups in the length of time between diagnosis and fertility preservation (17.4 ± 4.93 versus 16.4 ± 1.74 days). Oestradiol concentrations of breast cancer patients on the letrozole protocol remained much lower than those of patients on the antagonist protocol. 

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

KEYWORDS: breast cancer, fertility preservation, letrozole, non-hormone-dependent cancer

Introduction

Breast cancer is the most common malignancy among women worldwide (with the exception of non-melanoma skin cancers). The current estimated age-standardized incidence of breast cancer is 42.5–66.5 cases per

100,000 woman-years. In Europe, the incidence of breast cancer in premenopausal women over the past three decades was 30/100,000 (Glass et al., 2007). In Spain, the annual increase in incidence over the period from 1980 to 2004 was 1.7% (95% CI 1.4–2.1%) (Pollán et al., 2009).

Survival rates for breast cancer have risen in recent years, reaching 81–87%. Mortality rates have remained stable or decreased (Martos et al., 2009). Patient prognoses are quickly improving, which should be considered before beginning treatment; otherwise, the consequences can be devastating due the effects of chemotherapy on fertility. Many of these cancers affect patients in their reproductive years, and their childbearing is not yet finished. Approximately half of the demand for fertility preservation is from women with breast cancer (Letourneau et al., 2011; Lee et al., 2010). Fertility after treatment is a major concern for young women with breast cancer and many of them explore their options for preserving fertility (Partridge et al., 2004). A recent meta-analysis has shown that pregnancy after breast cancer improves survival (Azim et al., 2011). Women who have had breast cancer commonly avoid using hormonal treatments, and the professionals involved in their health care often have concerns about the use of hormones in these patients. The use of aromatase inhibitors may reduce the concentrations of oestradiol in these women (Azim et al., 2007; Oktay et al., 2006).

The aim of this study was to assess the effects of ovarian stimulation with letrozole by comparing women with breast cancer to those with non-hormone-dependent cancers and to prove that it should be used as an alternative for fertility preservation.

Materials and methods

This was a retrospective analysis of a prospective database of all patients with breast cancer and non-hormone-dependent cancer who were candidates for fertility preservation (before surgery or chemotherapy treatment) and were referred to the study centre by the oncologists and haematologists of the hospital. The study was conducted at the Reproduction Department of the Hospital del Mar, Universitat Autònoma de Barcelona and at the Centro de Infertilidad y Reproducción Humana, Spain from January 2009 to March 2011. The patients were recruited over 2 years. The patients included in the study signed an informed consent form for ovarian stimulation and oocyte vitrification.

Novartis, the manufacturer of Femara (letrozole), does not support its use for ovulation induction, but the Spanish Health Ministry has approved letrozole for compassionate use in breast cancer patients. Written informed consent to use letrozole was obtained from all of the patients before enrolment. The study was approved by the Ethics Committee of the hospital.

Inclusion criteria

The women enrolled in the study were between 18 and 37 years old and had no previous history of chemotherapy, exposure to any drugs that are toxic to the ovaries (such as methotrexate), ovarian surgery or infertility.

Baseline patient evaluation

The patients had a baseline evaluation during the early follicular phase (days 3–5 of their menstrual cycles) for concentra-

tions of FSH, oestradiol, anti-Müllerian hormone (AMH), hepatitis C virus, human immunodeficiency virus, hepatitis B surface antigen and syphilis serologies. An antral follicle count (AFC) was performed using transvaginal ultrasonography on the first or second day of the menstrual cycle.

Ovarian stimulation protocol

The ovarian stimulation protocol in the patients with non-hormone-dependent cancers consisted of the administration of daily recombinant FSH (Gonal F; Serono), beginning on the second or third day of menstruation. The starting dose of FSH was based on the AFC, age and weight of the patient and ranged between 150 and 225 IU/day. During ovarian stimulation, the plasma oestradiol concentrations were monitored every 48 h. To prevent a premature LH surge, 0.25 mg/day of a gonadotrophin-releasing hormone (GnRH) antagonist (Cetrotide; Merck-Serono) was administered when the oestradiol concentration was at least 500 pg/ml or the mean diameter of the follicles had reached 14 mm. Next, 0.3 ml of a GnRH analogue (Procrin; Abbot) was administered when at least three follicles had reached >17 mm in diameter. Transvaginal oocyte retrieval was performed 36 h after administration of the GnRH analogue.

For the breast cancer patients, this study followed a modified protocol used in the Controlled Ovarian Stimulation Treatment with Letrozole Supplementation Study (COST-LESS) GnRH agonists were replaced for GnRH antagonists to be able to start the new cycle. Also, a GnRH agonist was used as a trigger rather than HCG to prevent an increase in oestradiol concentrations (Oktay et al., 2005). The ovarian stimulation began on the second day of the menstrual cycle with the oral administration of letrozole (5 mg/day) and was continued until 48 h before oocyte retrieval. On the third day of letrozole administration, FSH was added at a dose of 150–225 IU/day, adjusted according to the patient's age, AFC and weight. As in non-hormone-dependent cancers, a GnRH antagonist was used to prevent an LH surge. When the diameters of at least three follicles were greater than 17 mm, ovulation was triggered with 0.3 ml GnRH analogue. After 36 h, the oocyte retrieval was performed transvaginally. Oocytes were assessed as mature after 90 min of retrieval and denuding.

The oocytes were cryopreserved by vitrification, using the closed system with the Irvine commercial kit and Cryotip (Irvine Scientific, USA).

After the oocyte retrieval, the patients were advised to restart the letrozole regimen until their oestradiol concentrations were <50 pg/dl.

Statistical analysis

The statistical tests were performed using Statistical Package for Social Sciences version 15.0 (SPSS, Chicago, IL, USA). The normally distributed continuous variables were compared using the independent-samples Student's *t*-test. The non-normally distributed continuous variables were analysed using the nonparametric Mann–Whitney *U*-test and the categorical variables were analysed using Fisher's Exact and chi-squared tests. $P < 0.05$ was considered statistically

significant. To obtain the oestradiol to oocyte ratio, the oestradiol concentration in pg/ml was divided by the number of vitrified oocytes.

Results

This study included 19 cancer patients. One group included 10 patients with non-hormone-dependent cancer, for which the haematology department of the study hospital referred nine patients with a lymphatic cancer diagnosis and the digestive oncologist referred one with a diagnosis of rectal cancer. The other group included nine patients with a diagnosis of breast cancer. Five patients had stage I disease and four patients had stage IIB disease, according to the tumour-node-metastasis (TNM) system. All but one breast cancer patient had positive oestrogen and progesterone receptors and two patients also showed Her2/neu positivity.

The groups were comparable in terms of age (28 ± 4.13 versus 32 ± 2.87 years) and gravida. All of the patients reported normal menstrual cycles before chemotherapy. The mean baseline FSH concentrations were within normal limits in the two groups, as were the AMH concentrations (Table 1). All patients were negative for hepatitis C virus, human immunodeficiency virus, hepatitis B surface antigen and syphilis serologies.

The mean duration from the cancer diagnosis to oocyte retrieval was 24.3 ± 6.79 days in the non-hormone-dependent cancer group versus 36.22 ± 12.30 days in the breast cancer group. The mean follow-up time between the first fertility preservation counselling session and the oocyte retrieval was 17.4 ± 4.93 days in the non-hormone-dependent cancer group and 16.4 ± 1.74 days in the breast cancer group. No statistically significant differences were found between the two groups. None of the patients experienced cycle cancellation due to spontaneous ovulation, poor response or ovarian hyperstimulation syndrome.

The characteristics of the ovarian stimulation cycles in the two groups are compared in Table 2. On average, the patients in the non-hormone-dependent cancer group needed 11.1 ± 1.79 days of ovarian stimulation, compared with 11.2 ± 0.66 days in the breast cancer group. The total dose of gonadotrophins used in the non-hormone-dependent cancer group was 2055 ± 565.90 IU/ml, compared with 2275 ± 484.60 IU/ml in the breast cancer group and the doses were not statistically significantly different. The total number of mature oocytes retrieved in the non-hormone-dependent cancer group was similar to that in the breast cancer group (15.4 ± 8.19 versus 16.3 ± 7.31).

The total number of vitrified mature oocytes was 14 ± 5.59 in the breast cancer group and 11.5 ± 6.65 in the non-hormone-dependent cancer group, although this difference was not statistically significant.

The oestradiol concentrations on the day of GnRH analogue administration were significantly higher in the non-hormone-dependent cancer group (1666.4 ± 739.42) than in the breast cancer group (829 ± 551.11) ($P = 0.006$). The mean oestradiol/oocyte ratio was also lower in breast cancer patients (55.5 ± 38.95 pg/ml per oocyte) than in patients with non-hormone-dependent cancer (127.61 ± 57.47 pg/ml per oocyte) ($P = 0.002$).

Discussion

This study showed that when using a modified COST-LESS protocol (Oktay et al., 2005), women with breast cancer exhibit significantly lower concentrations of oestradiol than do women with non-hormone-dependent cancers. The use of this protocol yields a number of vitrified oocytes similar to that observed with standard antagonist protocols in other cancer-affected women.

To minimize oestrogen exposure during ovarian stimulation, this study used an aromatase inhibitor (letrozole). The COST-LESS protocol (Oktay et al., 2005, 2010) showed lower peak oestradiol concentrations and outcomes, which were similar to those seen for standard IVF cycles in non-cancer patients, without increasing the rates of breast cancer recurrence. The mean oestradiol concentration in the letrozole group was half that of the non-hormonal-cancer group (829 ± 551.11 pg/ml versus 1666.4 ± 739.42 pg/ml, $P < 0.006$). The concentrations of oestradiol on the day of the GnRH or HCG trigger were associated with the concentration of ovarian response and the number of retrieved eggs. It is important to determine the oestradiol concentrations that are produced during oocyte retrieval. Peak oestradiol concentrations in a natural cycle can be as high as 225 ± 14.4 pg/ml (Marsh et al., 2011). The peak oestradiol concentrations per oocyte found in the breast cancer group were less than half the concentrations observed in the unstimulated cycles (55.5 ± 38 pg/ml). Preserving fertility by vitrifying oocytes in a spontaneous cycle would need 14 months to produce 14 vitrified oocytes, thereby cumulatively exposing the patient to approximately 3.346 pg/ml of oestradiol. In a single stimulation, this study obtained a mean of 14 oocytes with a mean oestradiol concentration of 829 ± 551.11 pg/ml, which is equivalent to the oestradiol exposure in 3.5 menstrual cycles.

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

Characteristic	Non-hormonal-dependent cancer (n = 10)	Breast cancer (n = 9)
Age (years)	28 ± 4.13	32 ± 2.87
Parity	1 ± 0.31	0
Antral follicle count	14.0 ± 6.33	12.6 ± 5.38
Anti-Müllerian hormone (ng/mL)	2.3 ± 1.27	2.5 ± 0.43
Baseline FSH (IU/ml)	4.3 ± 1.95	4.6 ± 1.89

Values are mean \pm standard deviation. There were no statistically significant differences between the two groups.

Table 2 Outcomes for ovarian stimulation and vitrification.

Outcome	Non-hormonal-dependent cancer (n = 10)	Breast cancer (n = 9)	P-value
Stimulation days	11.11 ± 1.79	11.22 ± 0.66	NS
Total FSH dose (IU/ml)	2055 ± 565.9	2275 ± 484.60	NS
Oestradiol on GnRH day (pg/ml)	1666.40 ± 739.42	829 ± 551.11	0.006
Total oocytes retrieved	15.40 ± 8.19	16.3 ± 7.31	NS
Total oocytes vitrified	11.50 ± 6.65	14.0 ± 5.59	NS
Immaturity rate (%)	11.8	10.7	NS
Oestradiol per oocyte (pg/ml)	127.61 ± 57.47	55.5 ± 38.95	0.002

Values are mean ± standard deviation unless otherwise stated. GnRH = gonadotrophin-releasing hormone; NS = not statistically significant.

The oestradiol concentrations encountered when using this protocol are low for three reasons. First, the peak oestradiol is lower in protocols that use aromatase inhibitors for ovarian stimulation (Oktay et al., 2005; Goswami et al., 2004; Verpoest et al., 2006). Second, the antagonist protocol also reduces the concentrations of oestradiol in oncological and infertile patients (Al-Inany et al., 2011). Finally, triggering with GnRH analogues has been shown to yield lower oestradiol concentrations compared with the HCG protocol (Oktay et al., 2010). Following the findings of the study of Oktay et al. (2010), GnRH was used to trigger ovulation, to avoid increasing oestradiol concentration and to minimize ovarian hyperstimulation syndrome (OHSS). The use of GnRH analogues improved the safety of the protocol without any cases of severe ovarian hyperstimulation (Ludwig et al., 2000; Bodri et al., 2010).

This study found no significant differences in the number of retrieved and vitrified oocytes. Although there were no statistically significant differences, the letrozole group had three more mature vitrified oocytes. One prospective (Garcia-Velasco et al., 2005) and two randomized (Goswami et al., 2004; Verpoest et al., 2006) trials have tested the use of letrozole in IVF protocols. Letrozole resulted in a significantly higher number of oocytes retrieved (Garcia-Velasco et al., 2005; Verpoest et al., 2006) and a significantly thicker endometrium (Verpoest et al., 2006), although these advantages were not consistently reflected in increased pregnancy rates.

The use of aromatase inhibitors in clinical practice must be considered carefully. At the moment, none of these agents are indicated for inducing ovulation in Europe or the USA. According to an abstract published in 2005 by the American Society for Reproductive Medicine (Biljan et al., 2005), the use of letrozole for inducing ovulation is discouraged. Novartis issued a statement advising that Femara had no indication in premenopausal women. The full-length paper corresponding to this abstract was not published owing to several methodological issues, such as comparing infertile women with healthy women, missing the follow up for 20 patients and a significantly younger control group.

A trial published in 2006 comparing the newborn safety of letrozole with that of clomiphene citrate did not support the concern that using letrozole for ovulation induction could be teratogenic (Tulandi et al., 2006). The only randomized trial assessing pregnancy outcomes after treatment with aromatase inhibitors in infertile women

involved 796 infertile women and was published in 2009 (Badawy et al., 2009). Consistent with the previous report by Tulandi et al., the authors failed to detect any differences in malformation rates among newborns after treatment with letrozole or anastrozole when compared with clomiphene citrate. The current study institution submitted a request to the Ministry of Health for the compassionate use of these drugs in all patients with breast cancer.

In some cancers, a delay in the start of chemotherapy is an important risk factor affecting survival. In other cancers, such as breast cancer, no effect on survival or recurrence has been found from initiating chemotherapy as late as 12 weeks after breast surgery (Lohrisch et al., 2006). The current study included women who were subjected to retrieval at the same time as their breast surgery. The patients contacted the department to arrange for measures to preserve their fertility on the day that the oncologists diagnosed their cancer. The average time between the oncologic diagnosis and oocyte vitrification was 3–5 weeks (24 days for non-hormonal cancer and 36 days for breast cancer patients). In other reports, the time from initial diagnosis to initiating ovarian stimulation was 42.6–71.9 days (Lee et al., 2010). The letrozole protocol does not increase the stimulation time; hence, it does not create any additional delays in initiating chemotherapy, which is beneficial to patients.

This study has certain limitations. It is a retrospective analysis of a prospective database. In addition, the number of included patients was small, as this institution is allowed to preserve fertility in only 12 women each year. The statistical comparisons of these data should take into account the small number of cancer patients included in each group. The strength of this study is that it appears to be the first to compare the fertility preservation between women with breast cancer and women with other tumours. However, the two different treatments for the two cancer groups make it difficult to make direct comparisons.

In conclusion, the COST-LESS protocol (with GnRH agonist triggering) used in women with cancer results in the same number of vitrified oocytes as a standard protocol and significantly decreased oestradiol concentrations compared with a standard protocol. More studies are needed to support the use of aromatase inhibitors in cancer patients and to eliminate the need to apply for compassionate use of these drugs.

Acknowledgements

The authors thank Marta Pulido, MD, for editing the manuscript and editorial assistance.

References

- Al-Inany, H.G., Youssef, M.A., Aboulghar, M., Broekmans, F., Sterrenburg, M., Smit, J., Abou-Setta, A.M., 2011. Gonadotropin-releasing hormone antagonists for assisted Reproductive technology. *Cochrane Database Syst. Rev.* 5, CD001750.
- Azim, A.A., Costantini-Ferrando, M., Lostritto, K., Oktay, K., 2007. Relative potencies of anastrozole and letrozole to suppress estradiol in breast cancer patients undergoing ovarian stimulation before in vitro fertilization. *J. Clin. Endocrinol. Metab.* 92 (6), 2197–2200.
- Azim Jr., H.A., Santoro, L., Pavlidis, N., Gelber, S., Kroman, N., Azim, H., Peccatori, F.A., 2011. Safety of pregnancy following breast cancer diagnosis: a meta-analysis of 14 studies. *Eur. J. Cancer.* 47 (1), 74–83, Epub 2010 Oct 11.
- Badawy, A., Shokeir, T., Allam, A.F., et al., 2009. Pregnancy outcome after ovulation induction with aromatase inhibitors or clomiphene citrate in unexplained infertility. *Acta Obstetrica et Gynecologica Scandinavica* 88, 187–191.
- Biljan, M.M., Hemmings, R., 2005. Brassard N The outcome of 150 babies following the treatment with Letrozole or Letrozole and gonadotrophins. *Fertil. Steril.* 84, pp. 0-231, Abstract 1033.
- Bodri, D., Guillén, J.J., Trullenque, M., Schwenn, K., Esteve, C., Coll, O., 2010. Early ovarian Hyperstimulation syndrome is completely prevented by gonadotropin releasing-hormone agonist triggering in high-risk oocyte donor cycles: a prospective, luteal-phase follow-up study. *Fertil. Steril.* 93 (7), 2418–2420.
- García-Velasco, J.A., Moreno, L., Pacheco, A., Guillén, A., Duque, L., Requena, A., Pellicer, A., 2005. The aromatase inhibitor Letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. *Fertil. Steril.* 84 (1), 82–87.
- Glass, A.G., Lacey Jr., J.V., Carreon, J.D., Hoover, R.N., 2007. Breast cancer incidence, 1980–2006: combined roles of menopausal hormone therapy, screening mammography, and estrogen receptor status. *J. Natl. Cancer Inst.* 99 (15), 1152–1161.
- Goswami, S.K., Das, T., Chattopadhyay, R., Sawhney, V., Kumar, J., Chaudhury, K., Chakravarty, B.N., Kabir, S.N., 2004. A randomized single-blind controlled trial of Letrozole as a low-cost IVF protocol in women with poor ovarian response: a preliminary report. *Hum. Reprod.* 19 (9), 2031–2035.
- Lee, S., Ozkavukcu, S., Heytens, E., Moy, F., Oktay, K., 2010. Value of early referral to fertility preservation in Young women with breast cancer. *J. Clin. Oncol.* 28 (31), 4683–4686.
- Letourneau, J.M., Ebbel, E.E., Katz, P.P., Katz, A., Ai, W.Z., Chien, A.J., Melisko, M.E., Cedars, M.I., Rosen, M.P. Pretreatment fertility counselling and fertility preservation improve quality of life in reproductive age women with cancer. *Cancer* 2011, doi:10.1002/cncr.26459. [Epub ahead of print].
- Lohrisch, C., Paltiel, C., Gelmon, K., Speers, C., Taylor, S., Barnett, J., Olivotto, I.A., 2006. Impact on survival of time from definitive surgery to initiation of adjuvant Chemotherapy for early-stage breast cancer. *J. Clin. Oncol.* 24 (30), 4888–4894.
- Ludwig, M., Felberbaum, R.E., Devroey, P., Albano, C., Riethmüller-Winzen, H., Schöler, A., Engel, W., Diedrich, K., 2000. Significant reduction of the incidence of ovarian hyperstimulation syndrome (OHSS) by using the LHRH antagonist Cetrorelix (Cetrotide) in controlled ovarian stimulation for assisted reproduction. *Arch. Gynecol. Obstet.* 264 (1), 29–32.
- Marsh, E.E., Shaw, N.D., Klingman, K.M., Tiamfook-Morgan, T.O., Yialamas, M.A., Sluss, P.M., Hall, J.E. Estrogen levels are higher across the menstrual cycle in African-American women compared with Caucasian women. *J. Clin. Endocrinol. Metab.* 2011.
- Martos, M.C., Saurina, C., Feja, C., Saez, M., Burriel, M.C., Barceló, M.A., Gomez, P., Renart, G., Alcalá, T., Marcos-Gragera, R. Accurately estimating breast cancer survival in Spain: cross-matching local cancer registries with the National Death Index. 2009 Rev Panam Salud Publica. 26, 51–4.
- Oktay, K., Buyuk, E., Libertella, N., Akar, M., Rosenwaks, Z., 2005. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and Letrozole for embryo cryopreservation. *J. Clin. Oncol.* 23 (19), 4347–4353.
- Oktay, K., Hourvitz, A., Sahin, G., Oktem, O., Safro, B., Cil, A., Bang, H., 2006. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. *J. Clin. Endocrinol. Metab.* 91 (10), 3885–3890.
- Oktay, K., Türkçüoğlu, I., Rodriguez-Walberg, K.A., 2010. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. *Reprod. Biomed. Online* 20 (6), 783–788.
- Partridge, A.H., Gelber, S., Peppercorn, J., Sampson, E., Knudsen, K., Laufer, M., Rosenberg, Przypyszny, M., Rein, A., Winer, E.P., 2004. Web-based survey of fertility issues in young women with breast cancer. *J. Clin. Oncol.* 22 (20), 4174–4183.
- Pollán M, Pastor-Barriuso R, Ardanaz E, Argüelles M, Martos C, Galcerán J, Sánchez-Pérez MJ, Chirlaque MD, Larrañaga N, Martínez-Cobo R, Tobalina MC, Vidal E, Marcos-Gragera R, Mateos A, Garau I, Rojas-Martín MD, Jiménez R, Torrella-Ramos A, Perucha J, Pérez-de-Rada ME, González S, Rabanaque MJ, Borrás J, Navarro C, Hernández E, Izquierdo A, López-Abente G, Martínez C. Recent changes in breast cancer incidence in Spain, 1980–2004. *J Natl. Cancer Inst.* 2009 Nov 18; 101(22): 1584–91. Epub 2009 Oct 26.
- Tulandi, T., Martin, J., Al-Fadhli, R., et al., 2006. Congenital malformations among 911 newborns conceived after infertility treatment with Letrozole or clomiphene citrate. *Fertil. Steril.* 85, 1761–1765.
- Verpoest, W.M., Kolibianakis, E., Papanikolaou, E., Smits, J., Van Steirteghem, A., Devroey, P., 2006. Aromatase inhibitors in ovarian stimulation for IVF/ICSI: a pilot study. *Reprod. Biomed. Online* 13 (2), 166–172.

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

Received 25 October 2011; refereed 3 February 2012; accepted 22 February 2012.