

A prospective case series of women with estrogen receptor-positive breast cancer: levels of tamoxifen metabolites in controlled ovarian stimulation with high-dose tamoxifen

E.M.E. Balkenende¹, T. Dahhan^{1,*}, S.C. Linn², N.G.L. Jager³, J.H. Beijnen³, and M. Goddijn¹

¹Centre for Reproductive Medicine, Department of Obstetrics and Gynecology, Academic Medical Centre, PO Box 22660, 1100 DD Amsterdam, The Netherlands ²Department of Medical Oncology, Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands ³Department of Pharmacy and Pharmacology, Slotervaart Hospital/The Netherlands Cancer Institute, Louwesweg 6, 1066 EC Amsterdam, The Netherlands

*Correspondence address. E-mail: t.dahhan@amc.uva.nl

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ABSTRACT: Controlled ovarian stimulation (COS) in women with estrogen receptor (ER)-positive breast cancer is potentially harmful because of the increase in serum estrogen levels. During COS for cryopreservation of oocytes or embryos, these women may receive high doses of tamoxifen (60 mg) to modulate the ER and prevent extra growth of estrogen responsive tumours during COS. However, it is unknown whether adequate serum concentrations of endoxifen, the most important metabolite of tamoxifen, can be reached. The aim of this study is to evaluate whether the tamoxifen dose used in a tamoxifen–COS combined schedule for women with ER-positive breast cancer is high enough to reach endoxifen levels that are considered therapeutically effective to inhibit breast cancer growth. The four women with ER-positive breast cancer who underwent COS for cryopreservation of oocytes were prospectively studied at the Academic Medical Centre, Amsterdam, the Netherlands. Throughout COS, blood samples were collected and tamoxifen and endoxifen levels were determined by a validated high-performance liquid chromatography tandem mass spectrometry assay. The four women with ER-positive breast cancer underwent a total of five COS cycles, while additionally using tamoxifen 60 mg daily. The tamoxifen and endoxifen levels showed a large variability between the women, with endoxifen levels during the whole period of ovarian stimulation varying between 3.96 and 41.0 ng/ml. The average number of vitrified oocytes was 11 (5–14). Therapeutically effective endoxifen serum levels can be reached when tamoxifen is used to counteract estrogen levels during COS for fertility preservation, but not in all women. Large variations of tamoxifen and endoxifen levels between the women were observed.

Key words: oocyte freezing / controlled ovarian stimulation / breast cancer / estrogen receptor / tamoxifen

Introduction

Breast cancer is the most common neoplasm found in women of reproductive age (Bray *et al.*, 2012). Most young breast cancer patients with a future wish to have children require lifesaving treatment with toxic side-effects on ovarian function. Cyclophosphamide, an alkylating agent commonly used in breast cancer, has known gonadotoxic effects that can lead to subfertility (Sonmezer and Oktay, 2006; Hulvat and Jeruss, 2009; Rodriguez-Wallberg and Oktay, 2010).

Cryopreservation of oocytes or embryos is a fertility preservation technique which can improve chances of offspring after breast

cancer treatment. Controlled ovarian stimulation (COS) is required in order to harvest a sufficient number of oocytes for either direct cryopreservation or fertilization followed by embryo cryopreservation. For a period of at least 2 weeks, women undergo treatment with high doses of follicle-stimulating hormone (FSH) for multifollicular growth, and concurrent ovarian suppression by down-regulation with GnRH-analogues. In response to daily gonadotrophin injections, women undergoing COS have a 2–3-fold higher peak estradiol (E2) level when compared with levels of the normal menstrual cycle (Barbieri, 2009; Strauss and Lessey, 2009). The theoretical risk of promoting breast cancer growth by COS in the case of estrogen receptor

(ER)-positive breast cancer has led to the use of adjusted COS protocols with the additional use of therapy regimens that can counteract the raised iatrogenic estrogen levels. This can be achieved by either blocking ERs by tamoxifen or by diminishing estrogen peak serum concentrations using aromatase-inhibitors (AI's). However, it remains unknown which COS protocol should be chosen in women with ER-positive breast cancer, to minimize the risk of adversely influencing the breast cancer outcome. Current literature does not supply evidence on which stimulation protocol is superior in terms of safety and IVF outcomes. One prospective study (Oktay et al., 2005) compared three adjusted stimulation protocols in 29 women with breast cancer. The women received tamoxifen alone, COS with tamoxifen or COS with letrozole. COS with tamoxifen resulted in statistically significant higher peak E2 levels (1182 ± 271 pg/ml) compared with letrozole and COS (E2 peak levels 380 ± 57 pg/ml) but there were no differences in breast cancer outcome after 18 months follow-up.

Tamoxifen is a non-steroidal anti-estrogen triphenylethylene derivative which, after oral administration, metabolizes a.o. to *N*-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen. These active metabolites, of which endoxifen is the most potent metabolite, selectively modulate the ER and suppress breast growth. However, the therapeutical efficacy (i.e. the protective effect of blocking the effect of raised estrogen levels during COS) and optimal therapeutic dose of tamoxifen for women with ER-positive breast cancer undergoing COS for oocyte- or embryo-cryopreservation is unclear. Since the terminal half life (time required to decrease the serum concentration with 50% of the steady state concentration) of tamoxifen is ~2 weeks, and steady-state concentrations are reached by only 2 months of administration, we wondered whether the higher tamoxifen dose used during COS would lead to adequate tamoxifen and, more importantly, endoxifen serum concentrations, since the most important tamoxifen metabolite is endoxifen.

The aim of this study therefore was to evaluate whether the tamoxifen dose used in a tamoxifenCOS combined schedule for women with ER-positive breast cancer is high enough to reach endoxifen levels that are considered therapeutically effective to inhibit breast cancer growth.

Materials and Methods

Women of reproductive age (18–40-year-old) who suffered from ER-positive breast cancer were eligible for inclusion if chemotherapy was indicated but not yet started. They were referred to the Centre for Reproductive Medicine of the Academic Medical Centre by their oncologists, nurse practitioners or were self-referrals, and recruited during the first consultation for fertility preservation. Women were excluded based on poor medical condition or the use of medication that might influence tamoxifen metabolism (e.g. fluoxetine) or if they were unwilling or unable to sign the informed consent form.

The study period was from April 2011 until May 2011. Women underwent down-regulation with a GnRH agonist in a long protocol with a mid-luteal start when time allowed for it. COS was started on cycle day 5 with recombinant FSH (rFSH) in daily doses ranging from 75 to 450 IU depending on the woman's age and the antral follicle count. In the case of time restraint, a short protocol with a simultaneous start to GnRH-a and rFSH was commenced between cycle day 1 and 3. Transvaginal ultrasound monitoring and measurements of luteinizing hormone (LH) and E2 were routinely performed during ovarian stimulation. Routine hormone levels

were assessed with radioimmunoassay (RIA, DPC, Los Angeles, CA, USA) for E₂, and electrochemiluminescence immunoassay (ECLIA, Cobas E immunoassay analyser, Indianapolis, IN, USA) for LH measurement. Follicular maturation was induced by 10 000 IU human chorionic gonadotrophin hormone (hCG) (Pregnyl®) when the majority of follicles reached 18–20 mm diameter as shown by transvaginal ultrasound. Transvaginal ultrasound guided follicle aspiration was performed 36 h later. Oocytes were cryopreserved at the metaphase II stage by vitrification.

Women were prescribed the use additional tamoxifen (60 mg, 3 oral tablets of 20 mg taken in one daily dose between 18:00 and 21:00 PM). Depending on how much time there was available to perform COS, women were prescribed to start tamoxifen as soon as they started GnRH analogues or FSH. Women received a diary to register the exact time of tamoxifen intake.

The levels of endoxifen and tamoxifen were analysed in serum samples acquired during routine blood testing during COS and on the day of ovum pick up. All serum gel tubes were coded and anonymized. The serum samples were collected in serum gel tubes and stored at -70°C until time of analysis.

An assay for the determination of tamoxifen (5–500 ng/ml) and endoxifen (1–100 ng/ml) from Teunissen et al. (2011) was used with slight modifications. Detection was performed on a triple-quadrupole MS/MS detector with an electrospray ionization source (API4000, AB Sciex, Foster City, USA) operating in the positive ion mode.

Data with regard to baseline clinical characteristics, the COS-cycles and outcomes in terms of number of oocytes retrieved and vitrified were collected. Graphs were made with SPSS version 19. The study protocol was approved by the Institutional Review Board of the Academic Medical Centre, Amsterdam and all women gave written informed consent before starting COS.

Results

A total of four eligible women with ER-positive breast cancer were included in this prospective case series. They all opted for oocyte cryopreservation prior to chemotherapy: three women before starting docetaxel, adriamycine and cyclophosphamide (TAC) cycles, and one woman before TAC and vincristine, epirubicin and cyclophosphamide (VEC) cycles. Baseline characteristics are presented in Table I. The mean age of the women was 26.5 years (range 24–28 years). Data with regard to the type of COS per cycle are presented in Table II. One woman underwent a long stimulation protocol and two underwent short stimulation protocols, and one woman underwent two long stimulation protocols. The average number of vitrified oocytes was 11 (range 5–14).

The tamoxifen and endoxifen levels showed a great variability between the women. Serum E2 levels increased during COS in all women and showed a normal pattern, with no major differences between the women (Fig. 1).

The endoxifen levels during the whole period of COS varied between 3.96 and 41.0 ng/ml. At the time of ovum pick up a large inter-individual variability in serum tamoxifen and endoxifen levels between the women was demonstrated (range 5.9–44.5 ng/ml; Figs 2 and 3). Increase in endoxifen levels observed in relation to duration of tamoxifen use (Fig. 4).

All patients were pleased with their decision to undergo oocyte cryopreservation. There were no complications during COS or ovum pick up for all four women in this study.

Table I Baseline clinical characteristics of four women with ER-positive breast cancer.

Baseline clinical characteristics	Case 1	Case 2	Case 3	Case 4
Age at start COS	27	24	28	27
Marital status	Married	Single	Married	Male partner
Previous pregnancies	0	0	1 LB	0
BMI (kg/m ²)	22.2	21.7	18.3	22.6
Histological findings breast tumour	IDC	IDC	IDC	IDC
Bloom–Richardson grade	2	3	Unknown	2
Hormone receptor status	ER+, PR+, Her2Neu–	ER+, PR+, Her2Neu–	ER+, PR+, Her2Neu–	ER+, PR+, Her2Neu+
Interventions before start COS	BCS	BCS + RT	BM	BCS
Interventions after start COS	RT + CT	RT + CT	AND + RT + CT	MX + CT
Type of chemotherapy	6 × TAC	6 × TAC	6 × TAC	3X VEC + 3X TAC
AFC	14	19	13	9
Serum E2 at start COS (nmol/l)	0.2	0.6	<0.1	1.5

AFC, antral follicle count; AND, axillary node dissection; BCS, breast-conserving surgery; BM, bilateral mastectomy; Her2Neu, Her2neu status; IDC, invasive ductal carcinoma; LB, live birth; MX, mastectomy; TAC, docetaxel, adriamycine and cyclophosphamide; RT, radiotherapy; CT, chemotherapy.

Table II Characteristics of four women undergoing COS cycles for purpose of oocyte vitrification.

Characteristics of COS cycles	Case 1	Case 2	Case 3–1	Case 3–2	Case 4
Stimulation protocol	Short protocol	Short protocol	Long protocol	Long protocol	Long protocol
FSH (days)	12	12	11	11	8
Dosage of rFSH/day (IU)	100/150 ^a	100	200	200	225
Total dosage of rFSH/cycle (IU)	1500	1200	2200	2200	1800
Number of follicles 18–20 mm at time of hCG injection	10	12	12	8	5
Serum E2 (nmol/ml) at time of hCG injection	8.6	12.1	9.9	9.3	5.0
Number of oocytes retrieved	14	19	15	13	6
Number of oocytes metaphase II	12	14	13	10	5
Number of oocytes vitrified	12	14	13	10	5
Complications COS/ovum pick up	None	None	None	None	None

^aThe dosage was increased on stimulation day 7. COS, controlled ovarian stimulation.

Patient descriptions

Case 1

A 27-year-old nulligravid woman presented with a left breast mass suggestive of malignancy after visualization by ultrasound and mammography. Core needle biopsy showed an ER-positive breast carcinoma. Breast-conserving surgery and sentinel-node procedure were performed, no axillary metastases were found. Radiotherapy, chemotherapy and subsequent possible future loss of fertility were discussed. She married shortly after being diagnosed with breast cancer. The woman referred herself for a fertility preservation consultation and was seen on menstruation cycle day 1, 2 weeks post-operatively. A short protocol of COS (simultaneous start of rFSH and GnRH-a) was started as well as additional tamoxifen 60 mg per day. The woman did not opt for another COS cycle. Chemotherapy was started 51 days after oocyte retrieval.

Case 2

A 24-year-old nulligravid woman palpated a large lump in her right breast for which a mammography and ultrasound of the breast and right axillary node was performed. Lumpectomy was planned 1 week later, followed by radiotherapy and chemotherapy. Histological findings revealed an ER-positive invasive ductal breast carcinoma with one axillary metastasis. The surgeon referred her to our centre, where she was seen 2 days post-operatively, to discuss options for fertility preservation. She had no male partner at the time of diagnosis. Her first cycle of COS was a long protocol of COS with tamoxifen. During this COS cycle, no extra blood samples were drawn as this cycle took place before the start of the current study.

She stopped using tamoxifen after ovum pick up and restarted 16 days later, with the initiation of her second COS cycle. As it was a late decision to start a second COS cycle, a short stimulation

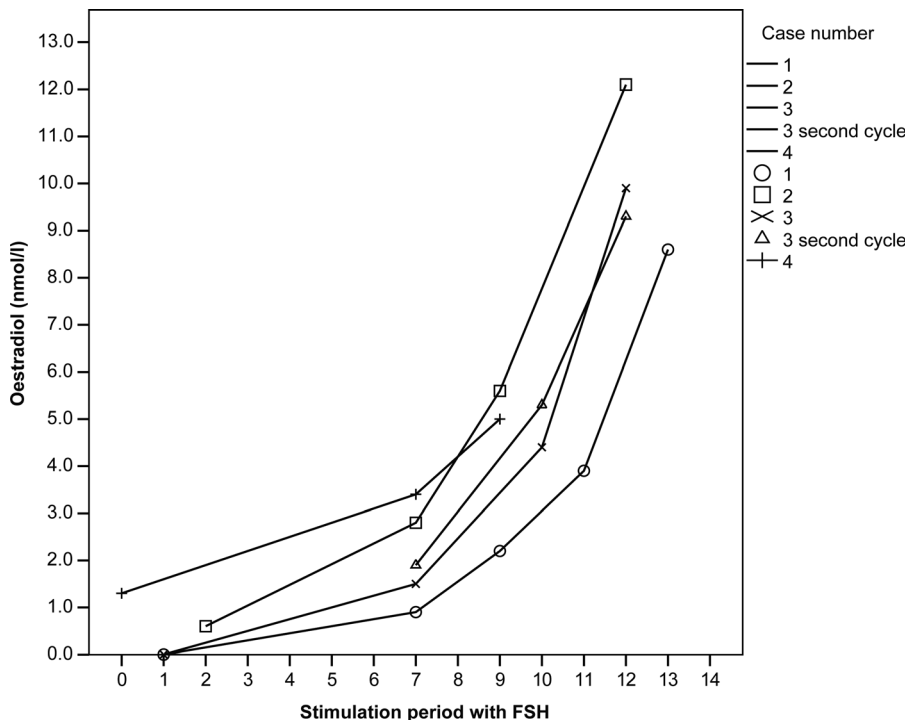


Figure 1 Serum estradiol levels during COS. $T = 0$ is day 1 of FSH administration. Dashes represent day of blood sampling and measurements of E2 levels. The final E2 measurements were before ovum pick up.

protocol and tamoxifen was started on cycle day 5. The woman reported misuse of tamoxifen in her diary and had used 30 mg in the luteal phase preceding the start of rFSH. On stimulation day 10, the dosage was corrected to 60 mg tamoxifen daily. Radiotherapy was continued until 5 weeks after first ovum pick up. Chemotherapy was initiated 54 days after the second oocyte retrieval.

Case 3

A 28-year-old woman, who gave birth to a child in 2010, palpated a lump in her left breast for which a mammography and ultrasound were performed. Core biopsy showed an ER-positive invasive ductal breast carcinoma. Bilateral mastectomy was planned, followed by gonadotoxic chemotherapy. The patient asked for a referral to our centre, for information about fertility preservation techniques. This married woman had a strong wish to vitrify oocytes because of her wish for autonomy in the course of fertility preservation. A long COS protocol with tamoxifen was started. After ovum pick up 15 oocytes were retrieved and 13 metaphase II oocytes were vitrified.

She opted for a second COS-cycle for oocyte vitrification, which commenced after surgical removal of axillary nodes. Tamoxifen was stopped after the last ovum pick up, for a period of 16 days. A long COS protocol with 60 mg tamoxifen was started on menstrual cycle day 5 after surgery. Seven days after her second ovum pick up she started with chemotherapy.

Case 4

A 27-year-old nulligravid woman palpated a small lump in the right breast. Visualization by ultrasound and mammography was performed

4 weeks later and showed malignant features. Histological findings revealed an ER-positive invasive ductal carcinoma with no metastasis in axillary nodes. She was advised to undergo mastectomy, followed by chemotherapy.

She was referred to our centre by her surgeon, and had had a male partner for 10 years. A long COS protocol with 60 mg tamoxifen was started. This woman started tamoxifen in the luteal phase (cycle day 23) together with the initiation of GnRH agonist. On cycle day 5 of the next cycle, transvaginal ultrasound showed two cysts in the right ovary. GnRH agonist was continued and on cycle day 15 bilateral transvaginal cyst puncture was performed and cytology showed no malignancy.

rFSH was started in the evening of cycle day 15. Seven days after ovum pick up the woman started chemotherapy.

Discussion

We assessed tamoxifen and tamoxifen metabolite levels in four women with ER-positive breast cancer who cryopreserved oocytes. Serum E2 levels increased during COS in all women, with no major differences between the women. The average number of vitrified oocytes was 11 (range 5–14). A large inter-individual variability in serum tamoxifen and endoxifen levels between the women at time of ovum pick up (range 5.9–44.5 ng/ml) was found. Of note, three out of four women achieved endoxifen levels considered adequate for ER inhibition (7 ng/ml; Madlensky et al., 2011).

Consistent with studies assessing pharmacokinetic effects of tamoxifen in the adjuvant setting, we found a large variability in analysed

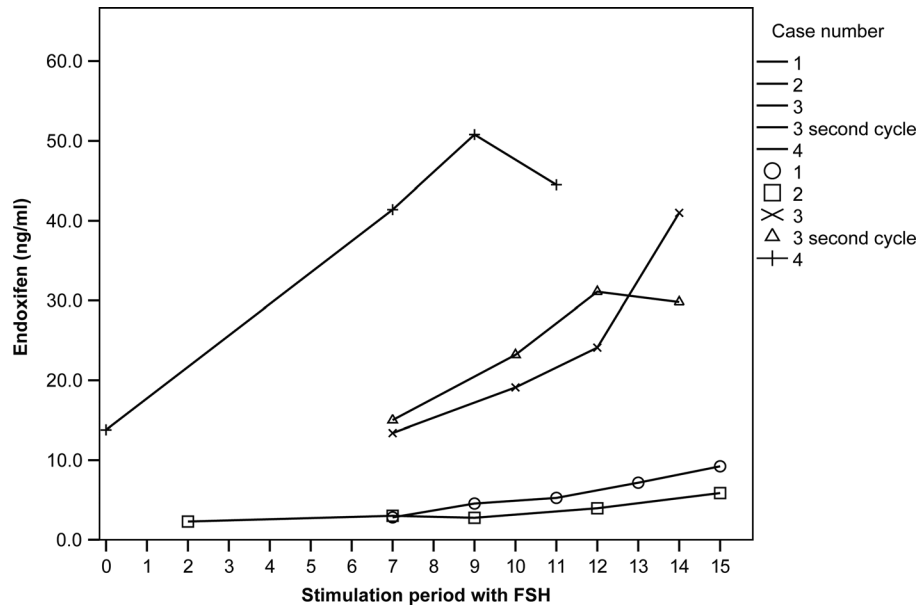


Figure 2 Serum endoxifen levels during COS. $T = 0$ is day 1 of FSH administration. All women started 60 mg tamoxifen together with FSH on stimulation day 1. Woman 4 started 60 mg tamoxifen in the luteal phase (cycle day 23) combined with GnRh-a (Decapeptyl). Last endoxifen measurements were performed at the day of ovum pick up.

endoxifen serum levels between women. Genotype variation, in particular the CYP2D6 genotype, has been found to be partly responsible for the large interpatient variability in endoxifen-levels (Borges *et al.*, 2010). Since only a minor fraction of the variation in endoxifen-levels can be explained by genotyping, analysis of endoxifen concentrations is more appropriate when assessing the pharmacokinetics of tamoxifen. Several studies support the idea that the therapeutic effect of tamoxifen in the adjuvant setting can be demonstrated when serum endoxifen levels reach a certain threshold (Jin *et al.*, 2005; Borges *et al.*, 2006; Madlensky *et al.*, 2011). Because the women in our study received high doses of tamoxifen during COS, the cut-off points of endoxifen levels described in current literature may be different from the cut-off point applicable for women in our study.

The pharmacokinetic action and biotransformation of tamoxifen when used during COS is unknown. By handing out a diary in which women reported the time and amount of tamoxifen intake, we minimized chances of inaccurate dose-related findings due to misuse of tamoxifen.

The long half life of tamoxifen might explain the observation that a prior cycle with tamoxifen, with discontinuation and restart before a next COS cycle (as in case number 3) contributes to higher levels of tamoxifen and endoxifen.

Our study has certain limitations. Because of the small case series, the interpretation of the results is difficult. Some women underwent a short COS protocol due to time restraints. This may have contributed to different outcomes in terms of serum tamoxifen and endoxifen levels and the number of vitrified oocytes.

Our centre uses tamoxifen as an additional agent to COS in women with ER-positive breast cancer. Another option to prevent the potentially harmful effect of high E2 levels is letrozole (A). Letrozole lowers

estrogen levels during COS (Oktay *et al.*, 2005). It has been found to suppress total-body aromatization by 98.9% in post-menopausal women (Dowsett *et al.*, 1995). Therefore, during COS, the additional use of letrozole to reduce peak E2 levels may be considered an alternative regimen to counteract the temporarily increased estrogen levels (Oktay *et al.*, 2006). In the absence of large randomized controlled trials evaluating the safety of adjusted COS protocols used in women with ER-positive breast cancer, insight into the pharmacokinetic action of these protocols is important. On the basis of these first results, we can conclude that therapeutically effective endoxifen serum levels, as when tamoxifen is used in the adjuvant setting, can be reached when tamoxifen is used to counteract estrogen levels during COS for fertility preservation. It is unknown whether the large interpatient variation of our study could influence the potential adverse effect of tamoxifen-protected COS on breast cancer outcome. A large prospective cohort study with a larger group of women is warranted.

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Authors' roles

M.G., S.C.L. and J.H.B. designed the study. E.M.E.B. and T.D. collected data and N.G.J. performed tamoxifen metabolite analyses. E.M.E.B.

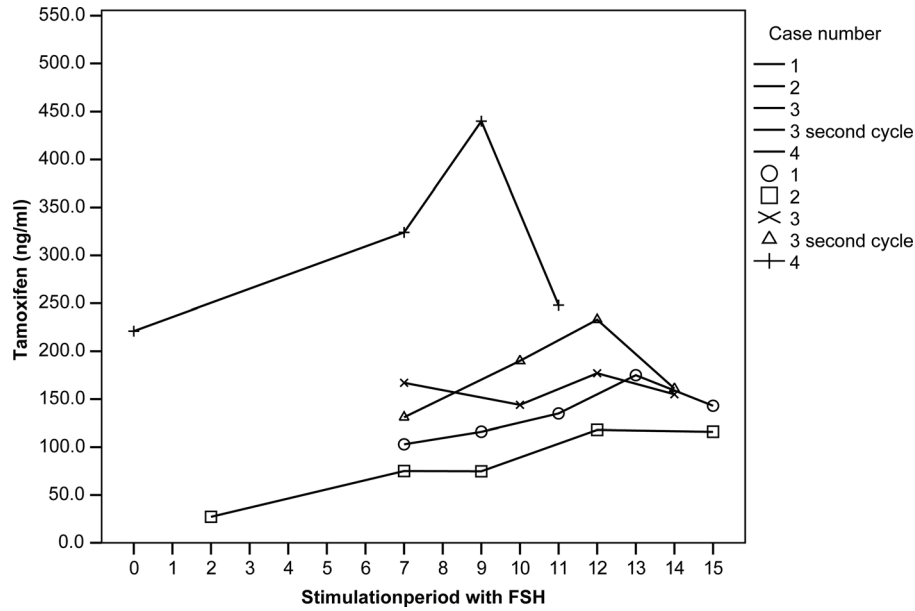


Figure 3 Serum tamoxifen levels during COS. $T = 0$ is day 1 of FSH administration. Case number 4 started 60 mg tamoxifen on cycle day 23, together with the initiation of GnRH-a (Decapeptyl). All other cases started 60 mg tamoxifen together with FSH on stimulation day 1. Last endoxifen measurements were performed at the day of ovum pick up.

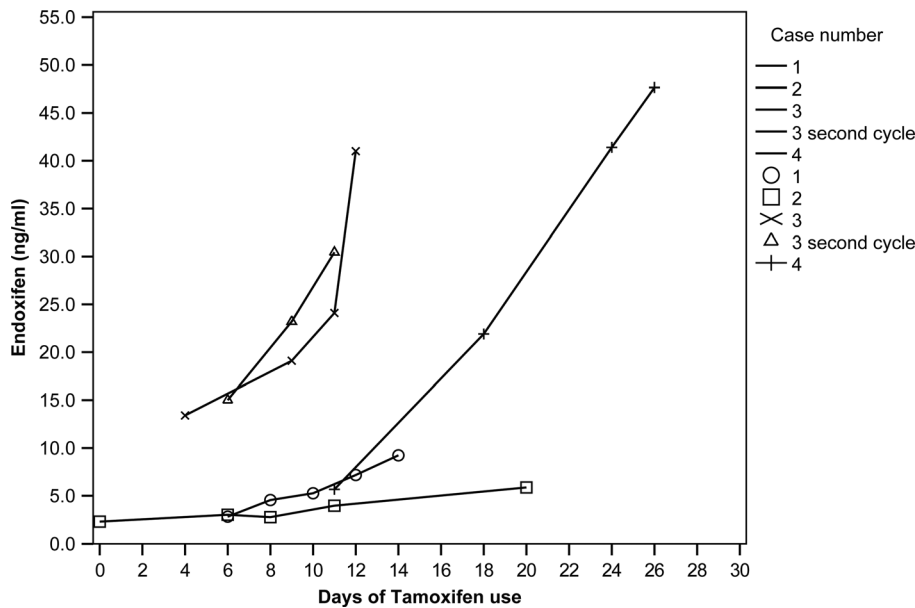


Figure 4 Serum endoxifen levels and days of tamoxifen use. $T = 0$ is day 1 of FSH administration. Case number 3 had restarted tamoxifen after withdrawal of 18 days.

and T.D. drafted the manuscript. M.G. supervised the study. All authors critically revised the manuscript and approved the final version of the manuscript.

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Conflict of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper. The authors declare that they have no competing interest.

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