GnRH agonist therapy as ovarian protectants in female patients undergoing chemotherapy: a review of the clinical data

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BACKGROUND: Cancer survival rates in young women are improving due to progress in treatment. This includes aggressive chemotherapy, a treatment that often poses a threat to fertility. GnRH agonists were proposed as ovarian protectors during gonadotoxic therapies. This study was undertaken in order to determine the clinical evidence concerning this issue. METHODS: The medical literature was searched for studies that reported on ovarian function after the administration of GnRH agonists concomitant with chemotherapy. Twelve studies met the predetermined selection criteria. RESULTS: Data on ovarian function were obtained for 579 women who received chemotherapy. Among 345 women who received GnRH agonist co-treatment, ovarian function was preserved in 91% and 9% had premature ovarian failure. In 234 women who did not receive GnRH agonist co-treatment, ovarian function was preserved in 41% and failed in 59%. Only two of the studies were randomized. The control and the GnRH agonist groups differed in several important characteristics: the follow-up times were not equal, different treatment protocols were utilized and end-points were poorly defined and inconsistent between the studies. CONCLUSIONS: The effectiveness of GnRH agonists as fertility-preserving agents is debatable. A thorough literature search has found insufficient evidence to show that GnRH agonist co-treatment is effective in protecting the ovary from the damage of chemotherapy. A large randomized controlled trial with adequate follow-up is needed.

Keywords: female infertility; GnRH agonist; ovarian function

Introduction

Cancer is a common ailment, not rare even in young women. It is estimated that 1.4 million new cases of cancer were diagnosed in the year 2005 in the USA, of which 4% or ~55,000 cases were patients under the age of 35 years (Lee et al., 2006). One in every 51 women will be diagnosed with cancer before the age of 40. Mortality from cancer is declining (Jemal et al., 2005). Significant progress in the past 25 years has been made, particularly in the pediatric population suffering from cancer (Ross and Olshan, 2004). Today, 1 out of every 900 Americans between the ages of 20 and 45 is a survivor of childhood cancer (Burns et al., 2006). Improved survival rates are due to progress in cancer treatment, including aggressive chemotherapy. Unfortunately, this treatment is often connected to an increased risk for chronic health conditions such as endocrinopathies including ovarian failure (Oeffinger et al., 2006). Reports on the toxicity of the chemotherapeutic drugs began to appear more than 30 years ago (Uldall et al., 1972; Warne et al., 1973; Rose and Davis, 1977; Chapman et al., 1979). Since then, many reports have documented the adverse effects chemotherapy has on the female gonads, with a wide variability between the different studies. The rate of post-chemotherapy amenorrhea has been reported to vary from 14% to 100% (Bines et al., 1996; Elis et al., 2006; Sonmez and Oktay, 2006). The degree of damage is most dependent on the age of the women at the time of therapy (Chapman et al., 1979; Whitehead et al., 1983; Kreuser et al., 1987; Rivkees and Crawford, 1988; Bines et al., 1996; Goodwin et al., 1999). Meirow found that among 168 female patients undergoing chemotherapy, 34% developed ovarian failure. The risk was higher for older women and those receiving alkylating agents (Meirow, 2000). Another study on 84 patients reached similar conclusions, in that 40% developed premature ovarian failure, in correlation with the...
patient’s age at the time of therapy (Franchi-Rezgui et al., 2003).

The effect of treatment on gonadal function in childhood cancer survivors is less prominent (Byrne et al., 1987). Ninety-four per cent of the girls under the age of 15 years receiving chemotherapy had preserved ovarian function (Ortin et al., 1990). This optimistic forecast needs to be taken cautiously since the follow-up period was 9 years, meaning that the true effect on long-term fertility was still unknown. Moreover, it was reported that survivors who regain ovarian function post-chemotherapy face the risk of premature menopause (Byrne et al., 1992; Lutchman Singh et al., 2005). In an animal study, it was shown that exposure to doses of chemotherapy strong enough to destroy half of the ovarian primordial reserve did not affect reproductive performance (ovulation, mating and pregnancy rates) shortly after treatment (Meirow, 1999). From this, it is deduced that clinical information such as regular menses at the completion of chemotherapy does not rule out ovarian damage, which may be clinically relevant in future years.

The mechanism by which chemotherapy causes ovarian damage is not completely understood. Histological examination of ovaries following chemotherapy demonstrated atrophy with a marked loss of primordial follicles and oocyte depletion (Himmelstein-Braw et al., 1978; Meirow, 1999; Meirow and Nugent, 2001; Oktem and Oktay, 2007). Alkylating agents’ effect on mammalian cells is through two mechanisms: cell killing and delay in cell cycle progression. At the sub-cellular level, macromolecules such as DNA, enzymes and other proteins are damaged through alkylation (Ataya and Moghissi, 1989). The primary targets of damage in the ovary are the pre-granulosa cells of the primordial follicle (Ataya et al., 1990; Meirow, 2000). Cell staining has demonstrated that apoptosis is the mechanism responsible for follicle loss (Meirow and Nugent, 2001). Injury to blood vessels and focal ovarian cortical fibrosis are additional pathways that are involved in ovarian injury (Meirow et al., 2007). Lately, Oktay has introduced another fascinating possible mechanism. It is based on the observation published by Johnson in Nature that proliferative germline stem cells that sustain oocyte and follicle production exist in the post-natal ovarian ovary (Johnson et al., 2004). The bone marrow was identified as the source of the oocyte-producing germ cells in adults (Johnson et al., 2005). Oktay proposed that chemotherapy damages not only primordial follicles but also unknown endocrine cells that produce the signals that recruit new germ cells from the bone marrow (Oktay, 2006). This hypothesis opens a broad field for future investigation.

In order to avoid ovarian damage and allow a normal reproductive life, major efforts are invested in developing fertility preservation techniques among cancer patients. These include: embryo cryopreservation, ovum (both mature and immature) cryopreservation, ovarian tissue cryopreservation, ovarian biopsy and GnRH analog administration (Donnez et al., 2000, 2004, 2006; Meirow and Nugent, 2001; Blumenfeld, 2003; Oktay and Sonmez, 2004; Revel and Schenker, 2004; Sonmez and Oktay, 2004; Falcone and Bedaiwy, 2005; Lobo, 2005; Meirow et al., 2005; Seli and Tangir, 2005; Demeestere et al., 2006; Marthom and Cohen, 2006; Oktay et al., 2006; Porcu and Venturoli, 2006; Rosendahl et al., 2006; The Practice Committee of the American Society for Reproductive Medicine, 2006; Maltaris et al., 2007).

GnRH was isolated and characterized in 1971. Analogues have been developed which have many clinical uses (Conn and Crowley, 1994). GnRH agonists affect the hypothalamus–pituitary–gonadal axis in two distinct mechanisms. As a single, short-term dose, they cause a sudden release of gonadotrophins from the pituitary gland (‘flare-up’), whereas with repetitive or long-term exposure, they bring about a down-regulation of the GnRH receptor causing complete ablation of the reproductive axis (Shalev and Leung, 2003).

Assuming that turning off the reproductive axis would make the ovary less vulnerable to cytotoxic damage, it has been proposed that GnRH agonists could be utilized as ovarian protectors during gonadotoxic therapies (Blumenfeld, 2007; Imai and Furui, 2007).

Animal studies have demonstrated a protective role for GnRH agonists against chemotherapy-induced gonadal damage. Administration of GnRH agonists to rodents causes inhibition in follicular recruitment, preventing their attaining the chemotherapy-sensitive stage. This presumably protects the female rats from chemotherapy-induced fertility reduction (Ataya et al., 1985, 1989; Ataya and Moghissi, 1989; Bokser et al., 1990; Ataya and Ramahi-Ataya, 1993). Letterie failed to demonstrate a benefit for GnRH agonists in a rat model (Letterie, 2004). In primates, Ataya demonstrated that three female rhesus monkeys receiving prior and concomitant treatment with luteinizing hormone-releasing hormone agonist retained significantly more primordial follicles than monkeys not receiving this treatment (Ataya et al., 1995).

There are several possible mechanisms through which GnRH agonists may protect the ovary during chemotherapy. Proposed mechanisms include protection via reduced levels of gonadotrophins, a direct influence of GnRH on the ovary and reduced blood flow to the ovary.

In the adult ovary, a cohort of primordial follicles is recruited to undergo a process where the majority will undergo atresia and one will become a dominant follicle. The basal follicular growth is independent of gonadotrophins (Gougeon, 1996; Meduri et al., 2003). It is possible that suppressing the gonadotrophin level with GnRH analogs preserves these follicles that have initiated growth and reached the gonadotrophin-dependent stage. However, growing follicles constitute <10% of all follicles and once growth has been initiated, they are destined either to become atretic or to ovulate (Sonmez and Oktay, 2004). Therefore, this explanation for a protectant mechanism for GnRH agonists could not explain a long-term effect.

GnRH receptors have been found in the non-primate ovary at all stages, but in the human ovary, they were detected only in the pre-ovulatory follicle and corpus luteum (CL) and not in follicles from the primordial to the early antral stage (Birnbaum et al., 1985; Janssens et al., 2000; Choi et al., 2006). In an in vitro experiment on human granulosa cells, it was shown that GnRH agonists protected the cells from damage caused from doxorubicin (Imai et al., 2007). The source of granulosa cells was mature follicles and it is unclear if the same effect would be found in primordial follicles. Since the ovarian reserve consists of primordial, intermediary and small primary follicles (Gougeon, 1996), it is doubtful if the mechanism for protecting the ovarian reserve during chemotherapy is through the GnRH receptor.

Another possibility is that there is a decline in ovarian blood flow during GnRH therapy, reducing the dose of chemotherapy reaching the ovary and therefore limiting the damage to the ovarian reserve. The effect of pituitary down-regulation on ovarian blood flow is still controversial. Some have shown a
decline in the ovarian flow (Engmann et al., 1999; Dada et al., 2001). Kitajima demonstrated, in a rat model, that ovarian stimulation causes an increase in ovarian weight, vascular permeability, vascular density and the vessel endothelial area and that all of these effects were inhibited by administration of GnRH agonist. Whether this is true in a human ovary not undergoing ovarian stimulation is unknown (Kitajima et al., 2006). In contrast, Yu showed that ovarian stromal blood flow measured by three-dimensional power Doppler ultrasound did not significantly change after treatment with GnRH agonist (Yu et al., 2004).

Other additional models which may explain a GnRH agonist effect include up-regulation of an intragonadal anti-apoptotic molecule such as sphingosine-1-phosphate (S1P) and protection of the undifferentiated germline stem cells (Blumenfeld, 2007). To the best of our knowledge, this proposed mechanism has not been tested under an experimental model. An additional assumption is an indirect influence through the transforming growth factor-β superfamily members. Numerous growth factors, many belonging to this group, are expressed by ovarian somatic cells and oocytes. An increasing body of experimental evidence supports their having key roles in multiple aspects of follicle development, including primordial follicle recruitment, granulosa and theca cell proliferation/atrophy, steroidogenesis, gonadotrophin receptor expression, oocyte maturation, ovulation, luteinization and CL formation. It is increasingly evident that considerable cross-talk exists between follicles at different developmental stages. The transition between a primordial to primary follicle (which is gonadotrophin-independent) might be under the regulation of ligands which originate from growing follicles (which are gonadotrophin-dependent) (Knight and Glister, 2006). Through that pathway, FSH (and GnRH agonist) might indirectly affect recruitment of primordial follicles. Presently, most of the evidence concerning the complex intraovarian control mechanisms have been generated using rodent models and there is a relative paucity of data on humans. Full understanding of the complexity of the intraovarian interactions is lacking and the protection mechanism offered is highly hypothetical and requires vast research efforts.

The clinical observation that pubescent girls are less susceptible to the ovarian damage of chemotherapy was the original rationale for employing GnRH agonists during chemotherapy. It was hypothesized that creating an artificial pubertal state might provide ovarian protection. This assumption may not be correct. Studies have shown that certain chemotherapy protocols induce ovarian failure even in pubescent girls (Teinturier et al., 1998). Meirow has suggested that chemotherapy damages the ovary regardless of the age during which it is administered. The greater apparent effect of chemotherapy in adults is due to the fact that the number of follicles in the ovary declines with age. When the total number of follicles is reduced below a certain threshold, the ovary fails and the patient becomes amenorrheic. In young females, with a large follicular reserve, the chemotherapy-induced loss of follicles might not be enough to cause immediate ovarian failure and amenorrhea. As time passes, the natural follicular loss is added to the chemotherapeutic reduction and result in premature ovarian failure. Indeed, studies have shown that in younger patients, many years after chemotherapy, the ovarian damage reveals itself through premature menopause (Byrne et al., 1992; Sklar et al., 2006). Older patients have already depleted ovarian reserve; therefore, the chemotherapeutic destruction deteriorates the follicular number below the threshold required to sustain ovarian function and the ovarian damage is evident in close proximity to the treatment (Meirow, 1999). If this is truly the explanation for the different clinical picture observed between younger and older patients, then the rationale for using GnRH agonist as an ovarian protectant is false.

On the other hand, GnRH agonists are widely available drugs, administration is simple and side effects are few; therefore, if proven effective, they would be an ideal fertility-preserving therapy.

This study was undertaken in order to review the clinical studies utilizing GnRH agonists as a gonadoprotectant during chemotherapy and to determine the evidence supporting its utility. Since there are few reports on fertility after chemotherapy concomitant with GnRH agonists, we evaluated studies which presented data on ovarian function tests as a surrogate end-point for fertility.

Materials and Methods
We carried out a literature search from 1966 to February 2008, without language restrictions, through the Medline database at the NCBI website (http://www.ncbi.nlm.nih.gov/sites/entrez). We added, as appropriate, articles found through the references of included articles. Using the search terms, ‘chemotherapy’, ‘gonadotoxicity’, ‘fertility preservation’, ‘fertility’ and ‘ovarian failure’ identified a total of 1,508,343 articles. We cross-matched the above terms with the phrases ‘GnRH agonist’ or ‘GnRH analog’ and were left with 991 titles. We reviewed these 991 titles and selected those articles that reported ovarian function after chemotherapy with GnRH agonist protection. Only abstracts written in English were included. In this manner, we located 25 articles (Waxman et al., 1987; Blumenfeld et al., 1996, 1999, 2000, 2002, 2008; Blumenfeld and Haim, 1997; Blumenfeld et al., 2001, 2002; Fox et al., 2001, 2003; Pereyra Pacheco et al., 2001; Recchia et al., 2002; Mardesic et al., 2004; Blumenfeld and Eckman, 2005; Dann et al., 2005; Franke et al., 2005; Somers et al., 2005; Del Mastro et al., 2006; Elis et al., 2006; Recchia et al., 2006; Castelo-Branco et al., 2007; Giuseppe et al., 2007; Potolog-Nahari et al., 2007; Huser et al., 2008). We excluded articles that met any of the following criteria: (i) articles reporting data which were reported once again in subsequent articles (eight articles were excluded, Blumenfeld et al., 1996, 1999, 2000, 2002; Blumenfeld and Haim, 1997; Blumenfeld et al., 2001, 2002; Recchia et al., 2002). (ii) Reports in which the data were presented only as an abstract and not a full peer-review article (Fox et al., 2001, 2003). (iii) Articles that combined treatment with GnRH antagonist and agonist (Mardesic et al., 2004; Potolog-Nahari et al., 2007). (iv) Articles where the data were not separated between those patients receiving GnRH agonist and those not receiving the agonist (Elis et al., 2006). Two articles by Blumenfeld were included since only some of the patients were identical, whereas others were unique to each article (Blumenfeld and Eckman, 2005; Blumenfeld et al., 2008). In total, 12 articles were included for review (Waxman et al., 1987; Pereyra Pacheco et al., 2001; Blumenfeld and Eckman, 2005; Dann et al., 2005; Franke et al., 2005; Somers et al., 2005; Del Mastro et al., 2006; Recchia et al., 2006; Castelo-Branco et al., 2007; Giuseppe et al., 2007; Blumenfeld et al., 2008; Huser et al., 2008) (Fig. 1).

Comparisons between the groups were done using Fisher’s exact test. \( P < 0.05 \) was considered statistically significant.
Results

Characteristics of the studies

Two studies were prospectively randomized (Waxman et al., 1987; Giuseppe et al., 2007) (Table I). Three studies were case series without control (Franke et al., 2005; Del Mastro et al., 2006; Recchia et al., 2006) and seven were case series with control (Pereyra Pacheco et al., 2001; Blumenfeld and Eckman, 2005; Dann et al., 2005; Somers et al., 2005; Castelo-Branco et al., 2007; Blumenfeld et al., 2008; Huser et al., 2008). The randomized controlled studies included 47 patients (22 in the study group and 25 in the control group). The case series without control included 133 patients and the case series with control included 510 patients (281 study and 229 control).

The disease and treatment

Among the 12 identified and selected studies, nine presented data of women with malignant hematological diseases (Hodgkin’s disease and non-Hodgkin’s lymphoma) (Table II). Two of the studies included patients with breast cancer and one study was of patients with systemic lupus erythematosus. The GnRH agonists used were tryptorelin (six articles), goserelin (three articles), leuprolide (two articles) and buserelin (one article). The chemotherapy protocols differ and in some studies radiotherapy was used as well.

Characteristics of the populations, follow-up and outcome measurements

The patients’ ages varied widely, between 14 and 50 years (Table III). There was also a great variability in the period which elapsed between the time chemotherapy was administered to the time ovarian reserve was determined (between less than a year and more than 8 years). Outcome measurements differ between studies and include menstruation, cycle regularity, hormone levels and ovarian sonography.

Ovarian function

In total, data on ovarian function were obtained for 579 women who received chemotherapy, including 345 women who received GnRH agonist co-treatment with chemotherapy and are referred to as the study group and 234 women who did not receive agonists and comprised the control group (Table IV).

Within the study group, ovarian function was reported as preserved in 314 women or 91%. Premature ovarian failure or persistent amenorrhea was reported in 31 women (9%). In the control group, ovarian function was reported as preserved in 97 women (41%) ($P < 0.01$). Premature ovarian failure or persistent amenorrhea was reported in 137 women (59%). The two prospective randomized studies (Waxman et al., 1987; Giuseppe et al., 2007) present data on 46 patients: 22 in the study group and 24 in the control group.

Table I. Characteristics of the 12 studies reviewed.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Evaluated patient no.</th>
<th>Prospective or retrospective study XY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Waxman et al. (1987)</td>
<td></td>
<td>PP</td>
</tr>
<tr>
<td>Pereyra Pacheco et al. (2001)</td>
<td>12 c</td>
<td>4 c</td>
</tr>
<tr>
<td>Blumenfeld and Eckman (2005)</td>
<td>75</td>
<td>82</td>
</tr>
<tr>
<td>Franke et al. (2005)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Dann et al. (2005)</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Somers et al. (2005)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Del Mastro et al. (2006)</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Recchia et al. (2006)</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Castelo-Branco et al. (2007)</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>Giuseppe et al. (2007)</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Blumenfeld et al. (2008)</td>
<td>65</td>
<td>46</td>
</tr>
<tr>
<td>Huser et al. (2008)</td>
<td>72</td>
<td>45</td>
</tr>
</tbody>
</table>

X study group: P, prospective; R, retrospective; ?, missing information.
Y control group: P, prospective; R, retrospective; ?, missing information; 0, empty group.
*In the study, there is another control group of premenarchal patient, not mentioned here.
The study was not designed to examine the influence of GnRH agonist but to check the ovarian function post-chemotherapy.
Table II. The diseases and treatments in the 12 studies reviewed.

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Disease</th>
<th>The GnRH agonist used</th>
<th>Treatment including alkylating agent (patients treated with Alk. agents/total patients)</th>
<th>BMT</th>
<th>Radiotherapy (No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxman et al. (1987)</td>
<td>HL, NHL</td>
<td>Buserelin</td>
<td>8/8</td>
<td>10/10</td>
<td>4/12</td>
</tr>
<tr>
<td>Pereyra Pacheco et al. (2001)</td>
<td>Hematological oncologic pathology</td>
<td>Leuprolide acetate</td>
<td>12/12</td>
<td>4/4</td>
<td>4/12</td>
</tr>
<tr>
<td>Blumenfeld and Eckman (2005)</td>
<td>HL, NHL-aggressive</td>
<td>Tryptorelin acetate</td>
<td>Not Mentioned</td>
<td>Not Mentioned</td>
<td>49</td>
</tr>
<tr>
<td>Franke et al. (2005)</td>
<td>HL</td>
<td>Tryptorelin acetate</td>
<td>4/5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Dann et al. (2005)</td>
<td>SLE</td>
<td>Goserelin acetate</td>
<td>7/7</td>
<td>6/6</td>
<td>26</td>
</tr>
<tr>
<td>Somers et al. (2005)</td>
<td>Leuprolide acetate</td>
<td>20/20</td>
<td>20/20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Del Mastro et al. (2006)</td>
<td>Breast cancer</td>
<td>Goserelin</td>
<td>28/28</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Recchia et al. (2006)</td>
<td>breast cancer</td>
<td>Goserelin</td>
<td>100/100</td>
<td>9/100</td>
<td>80</td>
</tr>
<tr>
<td>Castello-Branco et al. (2007)</td>
<td>HL</td>
<td>Tryptorelin</td>
<td>30/30</td>
<td>26/26</td>
<td>26/26</td>
</tr>
<tr>
<td>Giuseppe et al. (2007)</td>
<td>HL</td>
<td>Tryptorelin</td>
<td>29/29</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Blumenfeld et al. (2008)</td>
<td>HL</td>
<td>Tryptorelin acetate</td>
<td>40/65</td>
<td>36/46</td>
<td></td>
</tr>
<tr>
<td>Huser et al. (2008)</td>
<td>HL</td>
<td>Tryptorelin</td>
<td>72/72</td>
<td>45/45</td>
<td></td>
</tr>
</tbody>
</table>

HL, Hodgkin’s lymphoma; NHL, non-Hodgkin’s lymphoma; SLE, systemic lupus erythematosus; BMT, bone marrow transplantation.

Table III. Ages, follow-up duration and outcome measurements in the 12 studies reviewed.

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Age (years, mean/median)</th>
<th>Follow-up duration (years, mean/median)</th>
<th>Outcome measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Control</td>
<td>Study</td>
<td>Control</td>
</tr>
<tr>
<td>Waxman et al. (1987)</td>
<td>28.5</td>
<td>25.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Pereyra Pacheco et al. (2001)</td>
<td>16.8</td>
<td>17.8</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Blumenfeld and Eckman (2005)</td>
<td>25.5</td>
<td>26.7</td>
<td>NM</td>
</tr>
<tr>
<td>Franke et al. (2005)</td>
<td>21.4</td>
<td>&lt;1</td>
<td>Regular menstruation, FSH, LH, progesterone, estradiol, inhibin B</td>
</tr>
<tr>
<td>Dann et al. (2005)</td>
<td>25.6</td>
<td>26.5</td>
<td>5.34</td>
</tr>
<tr>
<td>Somers et al. (2005)</td>
<td>23.9</td>
<td>25</td>
<td>Up to age 40 years or POF diagnosis</td>
</tr>
<tr>
<td>Del Mastro et al. (2006)</td>
<td>38</td>
<td>1</td>
<td>Menstruation, FSH, estradiol</td>
</tr>
<tr>
<td>Recchia et al. (2006)</td>
<td>43</td>
<td>6.25</td>
<td>Regular menstruation, FSH, LH, progesterone, estradiol</td>
</tr>
<tr>
<td>Castello-Branco et al. (2007)</td>
<td>14–45*</td>
<td>30.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Giuseppe et al. (2007)</td>
<td>26.7</td>
<td>32.5</td>
<td>8</td>
</tr>
<tr>
<td>Blumenfeld et al. (2008)</td>
<td>23</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Huser et al. (2008)</td>
<td>29</td>
<td>32.5</td>
<td></td>
</tr>
</tbody>
</table>

*Range of ages including both the study and control group. NM, not mentioned; POF, premature ovarian failure.

control group. Combining the data for the two studies reveals that 18 (82%) resumed regular menstruation in the study group, whereas only 46% of the control group did so (P < 0.05). Giuseppe reported no significant difference in FSH, LH, inhibin B and anti-Mullerian hormone levels and in antral follicular count between the two groups. In each of the two randomized controlled studies, no significant difference between the two groups was found. The studies do not detail the number of women who desired pregnancy and their success rate. Rather, sporadic pregnancies are described for both study and control groups.

Major drawbacks of the studies

Waxman et al. (1987): the sample size is small. The upper age range in the control group is 46 years as opposed to 34 years in study group.

Pereyra Pacheco et al. (2001): the sample size is small. The follow-up in the control group is longer than in the study group. There are significant differences in the treatment protocol for the control and study groups. For example, bone marrow transplantation was done in 42% of the study group as opposed to 100% in the control group. There is no statistical analysis.

Blumenfeld and Eckman (2005): many details are missing (e.g., total alkylating dose and follow-up period). The control group included retrospective historical controls and concurrently treated patients.

Franke et al. (2005): the sample size is small. There is no control group. The follow-up period is short.

Dann et al. (2005): the sample size is small. The study is non-randomized. The follow-up period in the control group was longer than in the study group. The oldest patient in the study...
group was 30 years old versus 40 years in the control group. There is no statistical analysis.

Somers et al. (2005): the study is non-randomized. An unknown number of subjects used an estradiol patch during follow-up. Assessment of ovarian function was based on normal menses while FSH levels were studied only for patients with symptoms consistent with menopause.

Del Mastro et al. (2006): there is no control group. The follow-up period is short. Success was defined as resumption of menstrual activity or an FSH level below or equal to 40 IU/l. These FSH levels may not represent properly the ovarian reserve since 86% of the patients received tamoxifen while selective estrogen receptor modulators have been reported to decrease FSH levels (Reindollar et al., 2002; Ellmén et al., 2003; Cheng et al., 2004).

Recchia et al. (2006): there is no control group. The hormonal assessment done to measure ovarian function is unclear. There is no statistical analysis.

Castelo-Branco et al. (2007): the study is non-randomized. The ages of patients in the study group versus the control group are not reported separately. The follow-up duration is not mentioned.

Giuseppe et al. (2007): a difference exists in the follow-up duration between the study and the control groups. The time interval from the chemotherapy was a significant predictor of ovarian function.

Blumenfeld et al. (2008): the study is non-randomized. The control group is partially historical.

Huser et al. (2008): the study is non-randomized. The control group is partially historical. There is unequal distribution of the chemotherapy type between the two groups.

**Discussion**

In this review, we have described the clinical evidence regarding the use of GnRH agonists as ovarian protectors during chemotherapy.

On the basis of these numbers, it is tempting to draw far reaching conclusions regarding the advantages of GnRH agonists as a fertility protectant, but it should be remembered that despite these promising results, there are many drawbacks. The bulk of the patients included (643 patients) were from non-randomized studies whereas the randomized studies were too small to draw definitive results (47). Some of the studies have no control group at all, whereas, in others, the controls are historical. In reports in which the study group is followed prospectively and the control group retrospectively, the time between therapy and evaluation is much greater for the control group. The implications are that they are more likely to reach ovarian failure at the time of evaluation. The age range of the participants in the studies is broad. The treatment regimens (of the anti-neoplastic treatment and the GnRH agonist protocol) vary. Outcome measurements (including menstruation, cycle regularity, hormone levels and ultrasound appearance of ovaries) are not always defined and differ between the studies. The end-points of the studies (‘preserved ovarian function’ versus ‘ovarian failure’) are poorly defined and inconsistent between the studies.

The overall rate of ovarian failure in the control group when all studies were combined was 59%, which was influenced by two studies (with 173 patients) where more than 70% of the ovaries failed in the control group (Castelo-Branco et al., 2007; Huser et al., 2008). This number is much higher than the reported rate of ovarian failure for Hodgkin lymphoma survivors, which is only 32–37% (Meirow, 1999; Hauvik et al., 2006). An exaggerated rate of ovarian failure in the control group may partially account for the supposed benefit of GnRH agonists in the study group.

GnRH agonists have side effects such as hot flashes and decreased bone density (Adashi, 1994; Del Mastro et al., 2006). Furthermore, it is important to note that the GnRH receptor has been found in tumor cell lines. A possible effect GnRH analogs might have on the clinical progression of malignancies has not been studied (Bohmann et al., 2003; Lee et al., 2006). On the other hand, GnRH agonist co-treatment may prevent menorrhagia which is a serious complication in young female patients who suffer from severe thrombocytopenia during myelosuppressive treatment (Meirow et al., 2006; Quaas and Ginsburg, 2007).

Our thorough literature search has demonstrated that there are no prospective, randomized, controlled trials showing a significant effect of GnRH agonist in protecting the ovary from the damage of chemotherapy. Combining the data from the two randomized studies (Waxman et al., 1987; Giuseppe et al., 2007) showed a
statistically significant advantage in the groups receiving concomitant treatment with GnRH agonists. The bulk of these data come from the study by Giuseppe (Giuseppe et al., 2007) where none of the women receiving GnRH agonists became amenorrheic. The follow-up on the patients in the study group was shorter than on the controls (average 2.4 versus 5.9 years). As a consequence, many of the patients in the control group were already over 38 years old at the time of observation, whereas in the study group most were under 32 years old. Since the authors found that the time from the end of the chemotherapy was a significant factor influencing ovarian reserve, it cannot be concluded whether the difference in the ovarian test function was due to the agonist co-treatment or to the difference in the time passed since the chemotherapy. This drawback was acknowledged in the manuscript by Giuseppe.

After a systematic and strenuous analysis of the published literature, we feel that a conclusion cannot be drawn regarding the efficiency of GnRH agonists for fertility preservation in young female patients receiving chemotherapy. The drawbacks of the studies published so far are important enough that we cannot be sure that beneficial results found in the statistical analysis combining all studies (P < 0.01) (Table IV) or combining the two prospective randomized studies (P < 0.05) were not an artifact of faulty methodology rather than a true benefit for the therapy. We conclude that there is not enough evidence at this time to resolve this issue. That is not to say that the opposite has been proven either, i.e. that GnRH agonists do not help preserve fertility but rather that the time has come for a large, well-designed, prospective randomized study with a long follow-up period.

Recently Oktay et al. published a review on the same topic, reaching comparable conclusions (Oktay et al., 2007). A similar opinion was expressed by Moore and Theriault in their commentary on the review (Moore and Theriault, 2007). Young women and parents of minors coping with a diagnosis of cancer will undoubtedly focus on the treatment and survival. The medical team must have foresight and account for the patient’s quality of life after treatment. Fertility preservation is one of these issues. The clinician must present a realistic assessment of the risk to fertility and the options available to preserve it. The number of available fertility preservation techniques is growing; regretfully most of them are still experimental. We hope that this review has helped shed some light on this question, particularly concerning the use of GnRH agonists concomitant with chemotherapy.

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