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'Ovarian cryopreservation versus ovarian suppression by GnRH analogues: *primum non nocere*': Reply

Dear Sir,

We thank Professor Blumenfeld for raising this matter (Blumenfeld, 2004). We agree, as we concluded in our debate article, that patients should be presented with all the facts about all applicable options. In our centre, we counsel the patient regarding the uncertainty of the benefit of ovarian suppression, and do not recommend it as a proven beneficial method. In the debate article by Revel and Schenker (2004), the conclusion was similar to ours in that there is no consensus regarding the effectiveness of ovarian suppression. Because the benefits and long-term effects of GnRH analogue administration are unknown at the present time, it should only be given as a part of prospective research and under ethics committee-approved protocols. It cannot be assumed that GnRH analogue administration is without drawbacks. One drawback is that patients can be misled ino believing that this is a foolproof approach, and they may decide not to resort to more established approaches such as embryo cryopreservation. Apart from the cost and severe hot flushes, there could also be potential risks to the patient. When given to breast cancer patients prior to chemotherapy, the hypoestrogenic milieu can put cancer cells into the G_0 phase, making them less responsive to chemotherapy (Mullen *et al.*, 1991; Emons *et al.*, 2003). In fact, 50% of breast cancer cases express GnRH receptors, and it has been shown that the stimulation of these receptors by native GnRH and GnRH analogues can lead to protection of cancer cells

from chemotherapy-induced apoptosis (Emons *et al.*, 2003). Cyclophosphamide, the most potent gonadotoxic agent, has to be broken down into its toxic metabolites before it can exert its chemotherapeutic effects. No study has looked at whether GnRH analogue administration interferes with this process; it is theoretically possible that if there is a decrease in gonadal toxicity, it is due to reduced chemotherapeutic effectiveness. Another theoretical concern is the increased gonadotoxicity. Gonadotrophins induce a series of antioxidant enzymes called glutathione *S*-transferases (GSTs) (Toft *et al.*, 1997). These enzymes are present in granulosa cells of follicles of various stages in the human ovary (Rahilly *et al.*, 1991) and play a role in detoxifying chemotherapeutics (Gamcsik *et al.*, 1999). Ovarian suppression reduces the expression of these enzymes, in theory rendering follicles more vulnerable to the effects of chemotherapy.

Dr Blumenfeld presented a different interpretation of the study of Teinturier *et al.* (1998). In that report, while 12 of 21 subjects had clinical evidence of gonadal failure, another patient had laboratory evidence. Moreover, two patients had gonadotrophin levels at the upper limit of normal, bringing the percentage of subjects with diminished ovarian reserve to 71%. Interestingly, all 10 patients who received busulfan developed ovarian failure. As shown by Meirow *et al.* (1999) in a rodent model, immediate reproductive endocrine performance is not a sign that ovarian reserve is not altered. Because younger patients have a larger ovarian reserve, and because of the variation in chemotherapy regimens, they may not immediately suffer from ovarian failure. Eventually, every person who receives alkylating chemotherapy will suffer from ovarian failure. Since the median ages when chemotherapy was received and at follow-up were 7 (range 1.2–13 years) and 14.5 (range 11–21 years), respectively, it cannot be concluded that the remaining 29% of patients were not affected by chemotherapy. In fact, the major weakness of previous studies on the role of GnRH analogues has been the short duration of follow-up, compared with controls. In the study by Blumenfeld *et al*., 16 women with lymphomas who received GnRH analogue co-treatment for gonadal protection were compared with a historical control group. While all patients in the GnRH analogue group resumed menses, 61% in the control group experienced premature ovarian failure. However, mean follow-up was only 1.7 $\pm$ 1.0 years (range 0.5–4) in the study patients compared with 7.0 $\pm$ 4.9 years (range 1.5–8.0) in the controls (Blumenfeld *et al.*, 1996). Moreover, only four of 16 in the study group received cyclophosphamide, the principal gonadotoxic drug, compared with 10 of 18 in the control group. Of concern to us is that two of the patients in the group receiving GnRH analogue did not respond to chemotherapy and died, while all survived in the chemotherapy only group. The latter observation further increases our concern that GnRH analogues may be diminishing the effectiveness of chemotherapy. The report by Pereyra Pacheco *et al.* (2001) was a case series report. They reported a retrospective group of five pre-menarchal children (aged 3–7.5 years) who received polychemotherapy, and descriptively compared them with a 'prospective' group of 12 post-menarchal children (aged 14.7–20 years) who received GnRH analogues prior to chemotherapy and another retrospective group of four post-menarchal children (aged 15.9–20 years) who received chemotherapy without GnRH analogue co-treatment The duration of follow-up was different between the pre-menarchal and post-menarchal groups (18 versus 5 and 6 years, respectively). No statistical analysis was done to control for differences in groups or to compare outcomes. Dr Blumenfeld cited the presence of GnRH receptors on ovarian cancer lines as supporting evidence for the presumed gonadal protective effect of GnRH analogues. It is a basic biological fact that cancer cell lines are aberrant and express many genes that are not expressed in normal cells. Besides, the cell lines that were used in the study by Volker *et al.* (2002) were of surface epithelium origin and not germ cells. There is no evidence that human primordial and pre-antral follicles express GnRH receptors.

Blumenfeld argued that there is sufficient evidence for the presence of FSH receptors on human primordial follicles. The presence of mRNA does not mean that functional receptors are expressed on cells, and does not prove a 'physiological' role. In the study of Patsoula *et al.* (2003), oocytes were from antral follicles, not from primordial or pre-antral follicles. Also, when using the extremely sensitive technique of RT–PCR, positive results are questionable because of the inevitable contamination from surrounding mature granulosa cells. Moreover, in the study of Zheng *et al.* (1996), which focused on FSH mRNA expression in tubal epithelium, only a weak focal mRNA signal was seen by *in situ* hybridization in primordial follicles, and the data were not shown. When using *in situ* hybridization, because of the minute size and flattened shape of primordial pre-granulosa cells, it is technically challenging to distinguish background signals from signals in primordial follicles. By RT–PCR, we did not detect FSH receptor mRNA in isolated human primordial follicles (Oktay *et al.*, 1997). Moreover, when xenografted in hypogonadal-immunodeficient mice, human ovarian follicles continue to initiate growth (Oktay *et al.*, 1998). In patients with FSH receptor mutation, follicles continue to initiate growth (Meduri *et al.*, 2003). There has been no evidence for the presence of FSH receptor protein on primordial follicles. Primordial follicles continue to initiate growth through hypogonadal states such as pregnancy and use of oral contraception, and ovarian suppression by oral contraceptives does not prevent chemotherapy-induced gonadal damage (Whitehead *et al.*, 1983). As the authors of the study pointed out, increased depletion of primordial follicles in LH-overexpressing mutant mice does not indicate a direct effect of LH (Flaws *et al.*, 1997). In fact, in that study, the authors showed a reduction and not an increase in the fraction of follicles entering the growth pool (primary), and concluded that the effect of LH was indirect, and that their data did not prove that primordial follicles were gonadotrophin responsive. As was discussed in that report, LH receptors have never been detected in primordial or early pre-antral follicles, and the authors explained the loss of primordial follicles by the toxic effects of the local endocrine milieu stimulated by supraphysiologically high LH stimulation on stromal cells.

Animal studies are, by definition, prospective. In the study of Ataya *et al*., 1995, three primates were studied in each of the cyclophosphamide and cyclophosphamide + GnRH analogue

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groups. One animal in the cytoxan group died prematurely, further reducing the sample size. In the only prospective controlled study in humans, albeit small, no benefit of GnRH analogue co-treatment against chemotherapy-induced gonadal damage could be demonstrated (Waxman *et al.*, 1987).

Recent valuable work has challenged the notion that ovarian follicular reserve cannot be renewed in rodents (Johnson *et al.*, 2004). The belief that pre-pubertal girls are immune from chemotherapy damage is another old dogma, which gave rise to the hypothesis that ovarian suppression through the use of GnRH analogues should protect the gonads against chemotherapy. We should challenge this dogma by carefully analysing the pre-existing scientific data, and by conducting a prospective randomized study. As has been the case of diethylstilbestrol and HRT, too much confidence in descriptive and retrospective data may result in adverse effects in patients, which will be against the principle of '*primum non nocere*' (Beauchamp and Childress, 1994).

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