

# Efficacy of in vitro fertilization after chemotherapy

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**Objective:** To evaluate if in vitro fertilization (IVF) with embryo cryopreservation can be proposed to patients immediately after one or two regimens of chemotherapy.

**Design:** Retrospective study.

**Setting:** Academic research center and IVF unit.

**Patient(s):** Eleven young patients diagnosed with cancer between September 1999 and April 2003 who wanted to preserve their fertility via IVF.

**Intervention(s):** Stimulation and IVF before or soon after chemotherapy treatment.

**Main Outcome Measure(s):** The number and quality of embryos obtained after stimulation in cancer patients undergoing IVF before or soon after chemotherapeutic treatment.

**Result(s):** Four patients underwent IVF in the interval between two regimens of chemotherapy. Two of them had no follicular development; one underwent follicular puncture but no oocytes were retrieved; and, in one, six oocytes were harvested but only one good quality embryo was obtained. In the seven patients who underwent IVF before starting chemotherapy, between 4 and 11 embryos were obtained per patient, the majority being good quality embryos.

**Conclusion(s):** Because the efficacy of IVF is dramatically reduced after even one round of chemotherapy, IVF should be performed before chemotherapy. For those who require immediate chemotherapy, ovarian tissue cryopreservation and/or oocyte cryopreservation could be used before treatment. (*Fertil Steril*® 2005;83:897–901. ©2005 by American Society for Reproductive Medicine.)

**Key Words:** Chemotherapy, cancer, IVF, embryo quality, cryopreservation

Gonadal dysfunction is a common consequence of cytotoxic chemotherapy or radiotherapy. Before such potentially sterilizing treatments, three nonexclusive methods can be proposed to preserve female fertility: ovarian tissue cryopreservation, oocyte cryopreservation, and in vitro fertilization (IVF) with embryo cryopreservation. For women who have a steady partner, IVF with embryo cryopreservation is an applicable option. However, ovarian stimulation is time consuming, and chemotherapy cannot be delayed in most cancer patients. For this reason, some centers offer IVF and embryo cryopreservation in the interval between two chemotherapy regimens.

Chemotherapy induces acute follicular damage, leading to a reduction in the number of follicles and also to chronic damage to the quality of the follicles as they easily undergo atresia. Electron microscopy studies after chemotherapy have demonstrated the cellular features that are typical of early atresia in primordial follicles, such as intracytoplasmic vacuoles, multivesicular bodies, altered mitochondria, and myelinic-like structures (1). The mechanisms of follicular damage are only partially understood. It is well known that

chemotherapy affects dividing cells and thus kills growing follicles. Although primordial follicles do not undergo mitotic division, studies have stressed the role of apoptosis in their destruction by chemotherapy, with the first stages occurring in the granulosa cells (2–4).

Very little has been published on the efficacy and safety of IVF in patients who were previously treated for cancer; it is thus not easy to give them appropriate counseling. A study by Ginsburg et al. in 2001 (5) analyzed IVF outcome in patients who had previously been treated for cancer and subsequently wished to conceive. We found no published data, however, on patients undergoing IVF during or soon after chemotherapy or before bone marrow transplantation. We decided to conduct a retrospective analysis of our patients undergoing stimulation both before and after chemotherapy to determine the impact of one or two regimens of chemotherapy on IVF outcome with embryo cryopreservation.

## PATIENTS AND METHODS

### Patients

Eleven patients underwent stimulation and IVF just before or in the course of their chemotherapy treatment for cancer. The patients' ages at the time of oocyte retrieval varied between 22 and 33 years (mean: 27 years). The oncologic indications were three with leukemia (two acute myeloblastic [AML] and one acute lymphoblastic [ALL]), five with lymphoma (three Hodgkin's disease and two non-Hodgkin's lymphoma [NHL]), one with medullar aplasia (MA), one with ovarian

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borderline tumor (BOT) with microinvasive peritoneal implants requiring chemotherapy, and one with stage II ovarian carcinoma (OC II). In the woman with stage II ovarian cancer, one IVF attempt was authorized after a unilateral adnexectomy before chemotherapy and debulking surgery were initiated.

Between September 1999 and February 2001, four patients (Table 1: patients 1 to 4) underwent IVF stimulation after one ( $n = 1$ ), two ( $n = 2$ ), or three ( $n = 1$ ) chemotherapy regimens. They were referred by their oncologists to our IVF center after they had gone into remission, before any bone marrow transplantation. The ovarian stimulation protocol was started 4 weeks after the first chemotherapy regimen in patient 1, 14 weeks in patients 2 and 3, and 5 weeks in patient 4 (who received three chemotherapy cycles within this short time frame).

Between September 1999 and April 2003, seven patients underwent IVF and embryo cryopreservation just before starting chemotherapy (see Table 1: patients 5 through 11).

The mean age in the two groups was similar, and the groups could be compared.

### Stimulation Protocols

All the patients underwent a short stimulation protocol with down-regulation by either nasal busserelin spray or subcutaneous injections of triptorelin, 0.1 mg a day. On the third day following the start of down-regulation, ovarian stimulation was begun with daily injections of four ampules of purified follicle-stimulating hormone (FSH, Humegon; Organon, Oss, the Netherlands) as a starting dose, except in three patients who were given adapted stimulation protocols: patients 1 and 2 started stimulation with eight ampules a day and patient 7 with three ampules a day.

### In Vitro Fertilization

Oocyte recovery, fertilization, embryo culture scoring, and freezing were performed according to the protocols published by our team in 2001 (6). However, fertilization was always achieved by intracytoplasmic sperm injection (ICSI) to avoid the risk of nonfertilization because only one IVF attempt was authorized. All the embryos were frozen on day 2 or 3, depending on the day of pick-up but regardless of their quality.

### Statistical Analysis

The SPSS 11.5 program was used for statistical analysis (SPSS, Inc., Chicago, IL). One-way analysis of variance (ANOVA) was performed to compare mean scores between the two study groups.  $P < .05$  was considered statistically different.

## RESULTS

### Ovarian Stimulation

Four patients (patients 1 through 4) underwent IVF in the interval between two regimens of chemotherapy. Six oocytes were collected from the first patient (patient 1), of which four were metaphase II; after ICSI, only one embryo was obtained and cryopreserved. The second patient (patient 2) had only one growing follicle, and no oocytes were retrieved. In the other two patients (patients 3 and 4), stimulation was discontinued because of a complete lack of response (no follicular development) despite high-dose gonadotropin administration.

On the other hand, when IVF was performed before chemotherapy (patients 5 through 11), between 4 and 11 embryos were obtained for cryopreservation per patient. In these seven patients, a total of 88 oocytes were obtained after pick-up (between 8 and 25 oocytes per patient, for a mean of 12.6); this represents an almost 10-fold increase over the women who had already received chemotherapy, from whom only six oocytes had been retrieved (between 0 and 6 oocytes per patient, for a mean of 1.5). This constitutes a statistically significant difference ( $P < .05$ ).

The estradiol levels were significantly higher ( $P < .05$ ) in patients undergoing IVF before chemotherapy than after chemotherapy, reaching an average of 2658 pg/mL (range: 1202–6750 pg/mL) compared with just 203 pg/mL (range: 10–671 pg/mL), respectively at the time of  $\beta$ -hCG administration. The mean number of ampules required for stimulation per patient was 44 in the group undergoing IVF with embryo cryopreservation before chemotherapy compared with 84 in the group with previous chemotherapy, which was also a statistically significant difference ( $P < .05$ ).

### Embryo Stage

In the group undergoing IVF in the interval between two chemotherapeutic regimens, only one grade 2 embryo was obtained (patient 1).

In the group undergoing IVF before chemotherapy, the embryo scores ranged from grade 1 to 3 (no grade 4) (Table 2). A total of 45 embryos were obtained from the seven patients. Among these, 30 embryos (66.7%) were grade 2, representing a mean of 4.3 embryos per attempt, per patient. This is a statistically significant difference ( $P < .05$ ) from the other four patients, from whom only one grade 2 embryo was obtained (0.25 embryos per attempt, per patient).

When freezing was performed on day 2 (see Table 2: patients 5, 7, 9, 10), the majority of the embryos (92.3%) comprised at least three cells. When freezing was performed on day 3, 63.1% of the embryos had reached the five-cell to seven-cell stage, and 10.5% of them were observed to be compacting or compacted.

**TABLE 1****IVF outcome in cancer patients during (patients 1–4) or before (patients 5–11) chemotherapy.**

Patient	Age (y)	Pathology	Chemotherapy before IVF	E <sub>2</sub> at hCG (pg/mL)	Ampules used	Oocytes	Cryopreserved embryos
1	32	NHL	1 regimen <sup>a</sup>	671	102	6	1
2	22	AML	2 regimens <sup>b</sup>	121	78	0	0
3	26	AML	2 regimens <sup>b</sup>	<10	82	0	0
4	24	ALL	3 regimens <sup>c</sup>	<10	74	0	0
5	31	MA	0	2430	32	10	6
6	24	HL	0	2500	24	13	10
7	28	HL	0	2610	27	25	11
8	33	NHL	0	1202	24	8	5
9	25	BOT	0	6750	104	12	5
10	26	HL	0	1576	34	11	4
11	26	OC II	0	1540	63	9	4

Note: ALL: acute lymphoblastic leukemia; AML: acute myoblastic leukemia; BOT: borderline ovarian tumor; HL: Hodgkin's lymphoma; MA: medullar aplasia; NHL: non-Hodgkin's lymphoma; OC II: ovarian carcinoma, stage II.

<sup>a</sup> One regimen of ACVBP (adriamycin, cyclophosphamide, vincristine, bleomycin, prednisone).

<sup>b</sup> Two regimens of cytarabine and idarubicin.

<sup>c</sup> One regimen of COP followed by two regimens of COPADM (cyclophosphamide, oncovin, prednisone, adriamycin, methotrexate).

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## DISCUSSION

Cancer treatments may compromise fertility, and female cancer patients have few options for fertility preservation. In our country (Belgium), in vitro fertilization with embryo

cryopreservation is only appropriate for married women or those who have a partner. In our study, we have tried to establish whether IVF with embryo cryopreservation could be performed in the course of a chemotherapy treatment.

**TABLE 2****Embryo quality in cancer patients during (patients 1–4) or before (patients 5–11) chemotherapy.**

Patient	Chemotherapy before IVF	Day of freezing	Cryopreserved embryos	Embryo stage (cells) <sup>a</sup>					Embryo scoring (grade) <sup>b</sup>		
				2	3–4	5–7	>8	M	1	2	3
1	1 regimen	2	1	—	1	—	—	—	—	1	—
2	2 regimens	NA	0	—	—	—	—	—	—	—	—
3	2 regimens	NA	0	—	—	—	—	—	—	—	—
4	3 regimens	NA	0	—	—	—	—	—	—	—	—
5	0	2	6	—	2	4	—	—	1	4	1
7	0	2	11	1	4	6	—	—	1	10	—
9	0	2	5	1	3	1	—	—	—	5	—
10	0	2	4	—	4	—	—	—	—	4	—
6	0	3	10	2	4	3	1	—	—	—	10
8	0	3	5	—	—	2	2	1	—	5	—
11	0	3	4	—	1	1	1	1	—	2	2

<sup>a</sup> M = morulae, compacting and compacted embryos.

<sup>b</sup> Grade 1 embryo = top-quality embryo; grade 2 embryo = good quality embryo; grade 3 embryo = intermediate quality embryo; grade 4 embryo = poor quality embryo.

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Indeed, a delay in chemotherapy, necessary to conduct ovarian stimulation, would be unacceptable in some patients in view of the severity of the malignancy. In such cases, IVF with embryo cryopreservation could be proposed in the interval between two chemotherapy regimens.

The study by Ginsburg et al. in 2001 (5) is the only published study we encountered on IVF outcome in cancer patients after chemotherapy. In their retrospective study involving 15 women undergoing IVF after systemic cancer treatment, they reported a poorer response to gonadotropins than in women with locally treated cancers, and a significantly diminished ovarian response to ovulation induction compared with patients undergoing IVF before cancer treatment. It is apparent that all women with day-3 FSH levels over 15 were excluded from the study. Despite this exclusion criterion, the investigators concluded that a previous history of systemic chemotherapy diminishes the response to ovulation induction. However, the study does not analyze the number of regimens or type of chemotherapy that the patients received, or the interval between the chemotherapy and the IVF attempt.

In our study, we compared two groups of young cancer patients: women who underwent ovarian stimulation with embryo cryopreservation before chemotherapy (patients 7 through 11), and those who began ovarian stimulation immediately after one to three courses of chemotherapy (patients 1 through 4). This latter group comprised all those suffering from hematologic malignancies: two with acute myeloblastic leukemia (AML) and one with acute lymphoblastic leukemia (ALL). Indeed, in these patients, chemotherapy was started immediately after diagnosis, and their oncologist referred them to our department for IVF and embryo cryopreservation during their remission phase before bone marrow transplantation.

Patient 1 had received only one regimen of chemotherapy (ACVBP: Adriamycin, cyclophosphamide, vincristine, bleomycin, prednisone). Patients 2 and 3 (both with AML) had each received two regimens of cytarabine and idarubicin, and patient 4 (ALL) received three regimens comprising alkylating agents (cyclophosphamide, 300 mg/m<sup>2</sup>). Patients 3 and 4 showed a complete lack of ovarian response; in patient 2, one growing follicle was observed, but no oocytes were retrieved. The patient who had received only one regimen of chemotherapy (patient 1) was the only one from whom a single embryo was obtained for cryopreservation. Our study demonstrated that, even after one regimen of chemotherapy, the ovarian response was dramatically reduced despite high gonadotropin doses.

It would have been interesting to check ovarian status before chemotherapy, but, because of the emergency nature of the cases and the need to avoid any delay in the onset of chemotherapy, this evaluation was not possible. However, all of the patients were young (<33 years) and had regular ovulatory cycles.

In the group who had undergone ovarian stimulation before chemotherapy, all of the patients had statistically significantly more embryos to cryopreserve, with at least four embryos per patient. Their quality (66.7% good-quality embryos) was comparable with the mean quality of all embryos obtained in our IVF unit, the majority from noncancer patients (6). The embryo stage at the time of freezing was found to correlate with the day of freezing and corresponded well to normal developmental kinetics.

Chemotherapy is known to induce a marked loss in the number of follicles. Alkylating agents and vinblastine have a direct dose-dependent cytotoxic effect on primordial follicles during the course of therapy. In female mice mated 1 week after chemotherapy injections (cyclophosphamide, 75 mg/kg), Meirou et al. (7, 8) reported a statistically significant reduction in the number of pregnancy sacs and the proportion of corpora lutea resulting in viable fetuses. This may explain the very poor results of ovarian stimulation, providing evidence of the acute toxicity of the treatment.

Moreover, the Meirou study found the malformation rate increased at least 10-fold in the offspring of mice treated with cyclophosphamide 1 to 4 weeks before mating compared with the control group. Given that follicular growth from the primordial stage to the Graafian stage takes more than 3 months in women, a short interval between exposure to chemotherapeutic drugs and ovarian stimulation could involve oocytes being exposed to the drugs during their growth phase. This gives rise to genetic concerns about the quality of embryos obtained from oocytes harvested after recent exposure to chemotherapy (8).

Even if studies on pregnancy outcome in cancer survivors show no increase in the malformation rate when these patients achieve pregnancy several years after chemotherapy (9–11), these results cannot be extrapolated to women who undergo IVF for embryo cryopreservation immediately after chemotherapy. In the long-term survivors, growing follicles were at the quiescent stage during previous chemotherapy.

In conclusion, IVF efficacy is dramatically reduced after chemotherapy, even after only one regimen. For women whose cancer therapy can be delayed, IVF with embryo cryopreservation should be offered before chemotherapy and not after. For patients who require immediate chemotherapy (especially for hematologic malignancies, when a delay may be deleterious for the prognosis), ovarian tissue (or oocyte) cryopreservation (12–14) can be proposed before treatment as an alternative way of preserving fertility without delaying cancer treatment, especially in patients requiring heavy chemotherapy or those at risk of relapse.

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