

Oocyte cryopreservation in oncological patients

Eleonora Porcu^{*}, Raffaella Fabbri, Giuseppe Damiano, Rosita Fratto,
Susanna Giunchi, Stefano Venturoli

*Department of Obstetrics and Gynecology, Reproductive Medicine Unit, Infertility and IVF Centre,
University of Bologna, Via Massarenti 13, 40138 Bologna, Italy*

Abstract

The use of chemotherapy and radiotherapy in oncological patients may reduce their reproductive potential. Sperm cryopreservation has been already used in men affected by neoplastic disease. Oocyte cryopreservation might be an important solution for these patients at risk of losing ovarian function. A program of oocyte cryopreservation for oncological patients is also present in our center. From June 1996 to January 2000, 18 patients awaiting chemotherapy and radiotherapy for neoplastic disease were included in our oocyte cryopreservation program. Our experience documents that oocyte storage may be a concrete and pragmatic alternative for oncological patients. The duration of oocyte storage does not seem to interfere with oocyte survival as pregnancies occurred even after several years of gamete cryopreservation in liquid nitrogen.

© 2003 Published by Elsevier Ireland Ltd.

Keywords: Oocyte cryopreservation; Oncological disease; IVF

1. Introduction

Oncological patients undergoing chemotherapy or radiotherapy risk the wastage of their reproductive potential. Along with the advances in the management of malignancies, the concept of quality survival and the need of fertility potential preservation found a place. While for men the possibility of freezing sperms has been reality for many decades, for women fertility preservation is more complex and no alternatives were available until recently. Oocyte cryopreservation is potentially the best way to preserve female fertility but this technique was unreliable in the past as documented by the few pregnancies reported [1–3]. Recently methodological improvement made egg freezing more efficient with an increase in survival, fertilization and pregnancy rate [4–7]. Therefore we started a program of oocyte cryopreservation for oncological patients.

2. Materials and methods

2.1. Patients

From June 1996 to January 2000, 18 patients suffering from various malignancies were included in our oocyte

cryopreservation program. Breast cancer and ovarian cancer were excluded. Hormonal basal levels of FSH and LH before starting the protocol were normal. Multifollicular development was induced as previously reported [8].

Problems related to induction of super-ovulation were mainly related to the time lapse before the beginning of chemo-radiotherapy. Great efforts were made to reduce this time lapse to minimum.

According to the onset of the last menstrual period, we chose to start stimulation on the first day of menses with a flare-up GnRH analogs–gonadotrophin combination or, if enough time was available, to proceed with a standard GnRH analog long protocol with a single injection of depot analog followed by gonadotrophin stimulation.

With these protocols, oocyte pick-up was possible in a time lapse ranging from a minimum of 2 weeks to a maximum of 4 weeks. All oocytes retrieved with transvaginal ultrasound-guided pick-up were cryopreserved adopting a slow freeze/rapid thaw protocol.

2.2. Oocyte freezing protocol

Six hours after collection, the cumulus–corona complex of each oocyte was removed by briefly exposing it for 30–40 s to a buffered culture medium containing 40 IU/ml of hyaluronidase enzyme (type VIII, Sigma, Aldrich S. r. I., Milan, Italy) and aspirating it through hand-drawn glass pipettes. Afterwards, the oocytes were examined under an Olympus

^{*} Corresponding author. Fax: +390-513-05806.
E-mail address: porcu@med.unibo.it (E. Porcu).

Table 1
Oocyte cryopreservation in patient undergoing antitlastic therapy

Patients	Pathology	Age	Stimulation days	Estradiol	Number of FSH ampoules	Cryopreserved oocytes
1	CML	26	12	450	33	22
2	CML	27	9	1200	30	16
3	Craniopharyngiomas	14	8	750	28	18
4	Medulloblastoma	15	13	630	36	25
5	Myelofibrosis idiopathic	18	12	2820	31	28
6	Essential thrombocytosis	22	11	1150	38	17
7	Hodgkin's disease	17	12	950	36	21
8	CML	24	10	820	29	12
9	Multiple sclerosis	27	10	350	34	17
10	CML	21	9	630	32	12
11	Ewing's sarcoma	16	11	820	33	6
12	Wilms'tumor	19	13	615	39	15
13	CML	14	11	840	37	19
14	Hodgkin's disease	15	11	1230	34	11
15	CML	24	14	780	42	12
16	Ewing's sarcoma	18	13	1200	38	7
17	Hodgkin's disease	23	9	1900	28	9
18	CML	17	12	480	36	12
	Mean \pm S.D.	19 \pm 4	11 \pm 2	978 \pm 558	34 \pm 4	15 \pm 6

CML: chronic myelogenous leukemia.

IMT-2 inverted microscope at 400 \times magnification and assessed for their nuclear maturity. The oocytes were cryopreserved using a slow freeze/rapid thaw protocol. They were equilibrated for 10 min in phosphate-buffered saline (PBS) supplemented with 1.5 M 1,2-propanediol (PROH) and 30% Plasmanate (Diasint, Firenze, Italy). After equilibration, the oocytes were transferred in PBS supplemented with 1.5 M 1,2-propanediol, 0.2 M sucrose and 30% Plasmanate, loaded into plastic straws and placed in an automated Kryo II 10/17 biological vertical freezer (Planer Product Ltd., Datamed Milano, Italy) with the chamber temperature at 23 °C. The temperature was slowly reduced from 20 to -8 °C at a rate of -2 °C/min. Ice nucleation was induced manually by seeding. Then the temperature was gradually reduced to -30 °C at a rate of -0.3 °C/min and rapidly lowered to -150 °C at a rate of -50 °C/min. After 10 min of temperature equilibration, the straws were transferred into liquid nitrogen tanks and stored for 4 months.

2.3. Oocyte-thawing protocol

The straws were removed from liquid nitrogen, held at room temperature for 30 s and put into a 30 °C water bath for 40 s. The cryoprotectants were removed by stepwise dilution. The oocytes were equilibrated in 1.0, 0.5 M PROH solution for 5 min, then in 0.2 M sucrose and PBS for 10 min and finally transferred to a fresh culture medium at 37 °C in an atmosphere of 5% CO₂ for 3 h before ICSI.

2.4. Intracytoplasmic sperm injection

The oocytes were incubated at 37 °C in an atmosphere of 5% CO₂ until ICSI. Sperm selection was done by the

minipercoll technique. The sperm suspension was kept in the 37 °C incubator until the intracytoplasmic injection of the oocytes. The intracytoplasmic sperm injection technique was performed as previously reported [9].

3. Results

The mean age of patients was 19 \pm 4 (mean \pm S.D.). Days of ovarian stimulation were 11 \pm 2, number of FSH ampoules were 34 \pm 4 and estradiol levels were 978 \pm 558.

A mean of 15 \pm 6 mature oocytes were cryopreserved per patient (Table 1). No ovarian hyperstimulation syndrome developed. No therapy side effects or complications occurred.

4. Discussion

In woman treated for malignancies, amenorrhoea and early menopause are not uncommon [10]. While younger women may restore apparently normal ovarian activity, older women rarely recover this function. In addition, even young girls, after a period of normal menstrual cycles, may undergo premature ovarian failure because of a marked loss of primordial follicles [11]. Several preventive tools have been proposed to reduce the reproductive damage. Oral contraceptives [12] as well as GnRH analogs [13] and ovarian transposition [14] have been used with same efficacy.

Oocyte pick-up, fertilization and subsequent embryo storage have also been proposed for partnered women. However, ethical, moral and pragmatic consideration should be taken into account in this choice.

More promising and attractive appears the prospect of ovarian tissue cryopreservation [15–17]. The best choice would probably be oocyte storage before their damage.

In the past, oocyte cryopreservation was an unreliable technique because of the high risk of damaging several cell structures such as cytoplasmic membrane, zona pellucida, cortical granules, cytoskeleton and meiotic spindle during the whole process of freezing–thawing and by the cryoprotectants. An improvement of the freezing protocol and the introduction of intracytoplasmic sperm injection to fertilize thawed eggs were recently successfully experienced by our team, are achieved an increase in oocyte survival and fertilization rate. The safety of the technique was supported by basic studies [18] and confirmed by the birth of several healthy children [4,5]. In addition, oocyte freezing may be safely combined with other techniques such as testicular and epididymal sperm fertilization [6,7].

Our experience documents that oocyte storage may be a concrete, pragmatic alternative also for oncological patients. The duration of oocyte storage does not seem to interfere with oocyte survival as pregnancies occurred even after several years of gametes cryopreservation in liquid nitrogen.

The practical management of these women must consider several factors. (1) The age of the patients. In fact, super-ovulation is impossible in very young girls. (2) The type of malignancy. In estrogen-sensitive cancers the use of ovarian stimulation drugs should be considered carefully. (3) The time lapse available before chemo-radiotherapy.

In our experience, the egg freezing program appeared feasible in this first series of 18 girls. The super-ovulation was optimal and led to the collection and storage of an average of 15 oocytes. With our present survival and fertilization rates, these girls will have the chance of about two embryo transfers after their recovery from the malignancy.

In conclusion, mature oocyte cryopreservation is advisable when:

1. The malignancy is not estrogen sensitive.
2. A minimum of 2 weeks are available for super-ovulation.
3. The patient is young enough to ensure good quality of oocytes. The patient is not pre-pubertal.
4. The patient has normal ovarian function with normal follicular FSH levels.

5. Condensation

A program of oocyte cryopreservation may be a concrete possibility for future fertility options in oncological patients awaiting chemotherapy and/or radiotherapy.

References

- [1] Chen C. Pregnancy after human oocyte cryopreservation. *Lancet* 1986;i:884–6.
- [2] Chen C. Pregnancies after human oocyte cryopreservation. *Ann NY Acad Sci* 1987;541:541–9.
- [3] Van Uem JFHM, Siebzehnrubl ER, Schun B, Koch R, Trotnow S, Lang N. Birth after cryopreservation of unfertilized oocytes. *Lancet* 1987;i:752–3.
- [4] Porcu E, Fabbri R, Seracchioli R, Ciotti PM, Magrini O, Flamigni C. Birth of a healthy female after intracytoplasmic sperm injection of cryopreserved human oocytes. *Fertil Steril* 1997;4:724–6.
- [5] Porcu E, Fabbri R, Ciotti PM. Cycles of human oocyte cryopreservation and intracytoplasmic sperm injection: results of 112 cycles. *Fertil Steril* 1999;72(1):S2.
- [6] Porcu E, Fabbri R, Ciotti PM, Petracchi S, Seracchioli R, Flamigni C. Ongoing pregnancy after intracytoplasmic sperm injection of epididymal spermatozoa into cryopreserved human oocytes. *J Assist Reprod Genet* 1999;16(5):283–5.
- [7] Porcu E, Fabbri R, Petracchi S, Ciotti PM, Flamigni C. Ongoing pregnancy after intracytoplasmic injection of testicular spermatozoa into cryopreserved human oocytes. *Am J Obstet Gynecol* 1999;180(4):1044–5.
- [8] Porcu E, Dal Prato L, Seracchioli R, Fabbri R, Longhi M, Flamigni C. Comparison between depot and standard release triptorelin in in vitro fertilization: pituitary sensitivity, luteal function, pregnancy outcome and perinatal results. *Fertil Steril* 1994;62:126–32.
- [9] Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smits J, et al. High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 1993;8:1061–6.
- [10] Longhi A, Porcu E. Reproductive function in female patients treated with adjuvant and neoadjuvant chemotherapy for localized osteosarcoma of the extremity. *Cancer* 2000;89(9):1961–5.
- [11] Familiari G, Caggiati A, Nottola SA. Ultrastructure of human ovarian primordial follicles after combination chemotherapy for Hodgkin's disease. *Hum Reprod* 1993;8:2080–7.
- [12] Ramona M, Chapman B, Simon B, Sutcliffe SB. Protection of ovarian function by oral contraceptives in women receiving chemotherapy for Hodgkin's disease. *Blood* 1981;52(4):849–51.
- [13] Blumenfeld Z, Avivi I, Linn S, Epelbaum R, Ben Shahar M, Haim N. Prevention of irreversible chemotherapy induced ovarian damage in young women with lymphoma by a gonadotrophin releasing hormone agonist in parallel to chemotherapy. *Hum Reprod* 1996;11(8):1620–6.
- [14] Hadel K, Rich WM, Austin P, DiSaia PJ. The role of ovarian transposition in conservation of ovarian function in radical hysterectomy followed by pelvic radiation. *Gynecol Oncol* 1982;13:195–202.
- [15] Bahadur G, Steele SJ. Ovarian tissue cryopreservation for patients. *Hum Reprod* 1996;11(10):2215–6.
- [16] Gosden RG, Rutherford A, Norfolk R. Ovarian banking for cancer patients. *Hum Reprod* 1997;12(3):403–5.
- [17] Meirou D, Fasouliotis SJ, Nugent D, Schenker JG, Gosden RG, Rutherford AJ. A laparoscopic technique for obtaining ovarian cortical biopsy specimens for fertility conservation in patients with cancer. *Fertil Steril* 1999;71(5):948–51.
- [18] Gook D, Osborn S, Bourne H, Johnston W. Fertilization of human oocytes following cryopreservation normal karyotypes and absence of stray chromosomes. *Hum Reprod* 1994;9:684–91.