First French live-birth after oocyte vitrification performed before chemotherapy for fertility preservation

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Dear Editor,

We report the first French live birth after oocyte vitrification-thawing in a cancer survivor, and also the first live birth obtained by oocyte vitrification-thawing in a patient presenting premature ovarian failure (POF) after the treatment of a Hodgkin Lymphoma (HL). The patient gave her written consent for communication about the case.

The overall survival of patients with cancer has improved dramatically. Unfortunately, many treatments affect fertility; fertility preservation (FP) has become an important step in the management of young patients [1, 2]. In female patients, FP techniques indications depends on various parameters; vitrification of mature oocytes has emerged as an established method in the past years [3]. Currently, in the context of FP before cancer treatment, dozens of live births have been published after ovarian cortex cryopreservation and subsequent grafting [4]. Although oocyte vitrification is widely used in assisted reproductive techniques, it is probably insufficiently proposed to female cancer patients before gonadotoxic treatments. Indeed, to our knowledge, only six live births have been published after oocyte vitrification for fertility preservation.

A 29-year-old married childless patient was referred to our FP center in January 2013 for fertility preservation before the treatment of a grade IV Hodgkin lymphoma. The patient had been referred to hematologist in the context of multiple cervical and axillary lymph nodes involvement. These lymph nodes had appeared for weeks while she had suffered for months from night sweats and weight loss. The initial staging showed a disseminated stage IV disease, without bulk, solid organ compression or effusion. The patient was designated to receive six BEACOPP escalated cycles chemotherapy treatment. The dosing regimen was as follows: bleomycin 10 mg/m², etoposide 200 mg/m² (day 1–3), adriamycin 35 mg/m² (day 1), cyclophosphamide 1250 mg/m² (day 1), oncovin 1.4 mg/m² (max 2 mg) (day 8), procarbazine 100 mg/m² (day 1–7), and prednisone (40 mg/m²). Fertility preservation was recommended by the Institutional Clinical Board because of a high rate risk of infertility (01/14/2013). The patient was informed about FP techniques available for her situation: oocyte vitrification, IVF for embryo cryopreservation, and ovarian tissue cryoconservation (OTC) for subsequent grafting. In vitro maturation of oocytes for vitrification was not proposed because it is not available in our center. In this grade IV HL patient, the risk of ovarian invasion by malignant cells was not excluded, although a low incidence of malignant cells in ovaries of HL patients has been described [5]. Moreover, chances of spontaneous pregnancy were not null after BEACOPP escalated chemotherapy treatment. As OTC involves total or partial ovariectomy which could major ovarian failure, and as thawed ovarian tissue grafting is still considered as an experimental technique in France, she preferred a conservative FP technique. Oocyte vitrification was discussed to be a FP strategy adapted to their case. The patient received steroids to limit the HD symptoms and delay the treatment for controlled ovarian stimulation (COS).

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Table 1 Live births reported in Hodgkin lymphoma patients who preserved their fertility

Reference	Treatment	Age at FP (years)	Technique of FP (OTC/EC/OSF)	Storage time (months)	Technique of fertility treatment (TOTG/TET/OT)	Number of intrauterine gestational sacs	Number of live birth	Weeks of gestation	Weight of baby (g)	Sex of baby
Yang et al. (2007) [9]	76 Gy radiotherapy + ABVD 12 cycles + COPP + ASCT	27	OSF and embryo transfer in GC	72	OT	1	1	37	3062	Male
Dittrich et al. (2012) [10]	ABVD 6 cycles + 30 Gy radiotherapy + DEXA-BEAM 2 cycles + aBMT	27	OTC	60	TOTG	1	1	38	3360	Male

OTC ovarian tissue cryopreservation; EC embryo cryopreservation; OSF oocyte slow freezing; TOTG thawed ovarian tissue grafting; TET thawed embryo transfer; OT oocyte thawing; GC gestational carrier; ABVD adriamycin, bleomycin, vinblastine, and dacarbazine; COPP cyclophosphamide, vincristine, procarbazine, and prednisone; ASCT autologous stem cell transplantation; DEXA-BEAM dexamethasone, BCNU (carmustine), etoposide, Ara-C (cytarabine), and melphalan; aBMT autologous bone marrow transplantation

The patient's ovarian reserve was assessed in emergency by antral follicle count on intravaginal ultrasound (12 antral follicles), but AMH level was not assessed. Patient was on the 19th day of the cycle in luteal phase. Given the urgency to start COS, we induced a luteolysis by a single dose of GnRh antagonist (cetorelix, cetrotide 3 mg, Merck Serono, Darmstadt, Germany) followed 3 days after by a conventional antagonist protocol with menotropins 300 IU/day (Menopur, Ferring®, Saint-Prex, Switzerland). Cetorelix 0.25 mg (Cetrotide 0.25 mg, Merck Serono, Darmstadt, Germany) was introduced when the lead follicle was >12 mm to prevent premature LH surge [6]. To minimize abdominal discomfort after oocyte retrieval and to avoid the occurrence of ovarian hyperstimulation syndrome, final oocyte maturation was triggered on day 10 of stimulation when leading follicles were >17 mm by injection of a GnRh agonist (0.3 mg of Triptorelin, Decapeptyl® 0.1 mg, Ipsen Pharma, Signes, France). On the day of ovulation trigger, six follicles with a diameter of more than 17 mm were visible, and six follicles measured between 11 and 13 mm.

Follicular aspiration was performed after 36 h, and six cumulus-oocyte complexes were retrieved. They were incubated for 1 h at 37 °C, 5 % CO₂ in culture medium (FertiCult® IVF medium, Fertipro, Beernem, Belgium) then the cumulus cells were removed, revealing four metaphase II mature oocytes, which were vitrified according to the manufacturer's protocol (Vit Kit®- Freeze, Irvine Scientific) in High Security Vitrification straws (Cryo Bio System®). Although rescue in vitro maturation of denuded oocytes does not contribute to the outcomes in poor-responder cycles [7], in the light of maximizing the chances for this cancer patient with high risk of chemo-induced POF, the two remaining immature oocytes were incubated for 20 h in the same condition. This operation led to the late in vitro maturation of one more oocyte, which was vitrified the day after. The five vitrified oocytes were subsequently stored in liquid nitrogen.

The patient received the six cycles of BEACOPP esc. scheduled. She reached a complete response from the first interim positron emission tomography (PET).

Sixteen months after the end of treatment (August 2014), the patient was in complete remission but presented a POF with amenorrhea and climacteric syndrome with hot flushes. Sonography showed hypotrophic ovaries (10 × 15 mm) without antral follicles, and AMH level was low (0.3 ng/ml, Beckman Coulter®, Brea, USA). Plasmatic level of FSH was 68 UI/L and estradiol <15 pg/mL. A sequential hormone replacement therapy (hrt) by estradiol (2 mg per day, 28 days by month) and progesterone 200 mg (14 days by month) was started.

Twenty-two months after the end of treatment (February 2015), patient was always in complete remission and pregnancy was authorized by oncologists. In March 2015, artificial endometrial preparation was performed with oral estradiol

Table 2 Indications and live births obtained by oocyte vitrification-thawing in cancer patients

Reference	Type of malignancy	Age at FP (years)	Number of vitrified oocytes	Type of cryostorage (open/closed)	Storage time (months)	Number of intrauterine gestational sacs	Number of live birth	Weeks of gestation	Weight of baby (g)	Sex of baby
Kim et al. (2011) [12]	CML + aBMT	22	7	Open	109	1	1	35 + 3 days	2410	Male
Garcia-Velasco et al. (2013) [13]	NHL	31	4	Open	24	1	1	39	3440	Male
Alvarez et al. (2014) [14]	Ovarian cancer	28	14	Open	12	1	1	38	2650	Male
Da Motta et al. (2014) [15]	Breast cancer	36	28	Closed	72	3	1	At term	2970	?
Martinez et al. (2014) [16]	Breast cancer/breast cancer	30/33	5/3	Open/open	36/80	1/1	1/1	40/40	2850/3220	??

CML + aBMT chronic myeloid leukemia + allogenic bone marrow transplantation, NHL non-Hodgkin lymphoma

4 mg daily. After ultrasound diagnosis of endometrial thickness at 12 mm, vaginal progesterone was administered (600 mg daily) for an embryo transfer planned 4 days after.

The five vitrified oocytes were warmed according to the manufacturer's protocol (Vit Kit®-Thaw). The oocyte vitrified 24 h after oocyte pick-up did not survive; after 3 h of culture in Fercult® + 20 % Human Serum Albumin medium, the four intact oocytes were inseminated with the husband's semen using intra-cytoplasmic injection. Normal fertilization of four oocytes was assessed at 18 h. At day 3, four diploid embryos were obtained. Only one embryo was graded A, which did not fulfill our criteria of single embryo transfer. As the patient had no obstetrical contra-indication to twin pregnancy, two embryos (graded A/B) [8] were transferred into the uterine cavity under transabdominal ultrasound guide and one (graded B) was cryopreserved. Endometrial thickness on the day of transfer was 12 mm.

Fourteen days after embryo transfer, the serum HCG level was 695 IU/L. A transvaginal ultrasound was performed on the sixth gestational week to confirm pregnancy. HRT was maintained during the whole first trimester of pregnancy. In December 2015, at 37.5 weeks of gestation, elective induction of labor was decided for a moderate gravid hypertension and patient delivered a healthy girl weighting 3180 g.

We believe that it is still important to publish such results, in order to develop the use of oocyte vitrification as a fertility preservation technique.

After treatment of HL, very few live births were reported after fertility preservation in patients presenting POF [9, 10] (Table 1). Most of these patients underwent a chemotherapy conditioning regimen before a bone marrow transplantation. BEACOPP regimen contains alkylating agents that are well-known to induce ovarian damage and POF. Moreover, no protective agents are effective to prevent follicle loss [11].

Oocyte vitrification technique is widely used for IVF treatments, oocyte donation, and social fertility preservation; nevertheless, to our knowledge, only six live births are described in cancer patients [12–16] (Table 2). This could be explained by the delay between the cancer diagnosis and the remission, with the need of consent from oncologists for a pregnancy. Moreover, a controversy has emerged among oncologists about the potential risk associated with high levels of estradiol during the COS, in some types of cancer [2]. Nevertheless, high estradiol levels have not been associated to a poorer prognosis in Hodgkin lymphoma.

In HL female patient receiving COS for fertility preservation, a low ovarian response is commonly described [17]. Our case report suggests that even in a case of low ovarian response, COS should not be canceled in HL patients.

In conclusion, oocyte vitrification is a simple, safe, and efficient technique to preserve fertility. Cancer patients should be informed that their fertility potential is related to the

number of vitrified oocytes, but our case report shows that even a low number of cryopreserved oocytes can allow the achievement of a healthy live birth.

References

1. Naessén S, Bergström I, Ljungman P, Landgren B-M. Long-term follow-up of bone density, general and reproductive health in female survivors after treatment for haematological malignancies. *Eur J Haematol*. 2014;93(2):137–42.
2. Cobo A, Garcia-Velasco JA, Domingo J, Remohí J, Pellicer A. Is vitrification of oocytes useful for fertility preservation for age-related fertility decline and in cancer patients? *Fertil Steril*. 2013;99(6):1485–95.
3. De Vos M, Smits J, Woodruff TK. Fertility preservation in women with cancer. *Lancet Lond Engl*. 2014;384(9950):1302–10.
4. Donnez J, Dolmans M-M. Ovarian tissue freezing: current status. *Curr Opin Obstet Gynecol*. 2015;27(3):222–30.
5. Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: a review of the literature. *J Assist Reprod Genet*. 2013;30(1):11–24.
6. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. *Fertil Steril*. 2013;100(6):1673–80.
7. de Braga DP AF, de Figueroa R CS, Ferreira RC, Pasqualotto FF, Iaconelli A, Borges E. Contribution of in-vitro maturation in ovarian stimulation cycles of poor-responder patients. *Reprod Biomed Online*. 2010;20(3):335–40.
8. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod Oxf Engl*. 2011; 26(6):1270–83.
9. Yang D, Brown SE, Nguyen K, Reddy V, Brubaker C, Winslow KL. Live birth after the transfer of human embryos developed from cryopreserved oocytes harvested before cancer treatment. *Fertil Steril*. 2007;87(6):1469. e1–4.
10. Dittrich R, Lotz L, Keck G, Hoffmann I, Mueller A, Beckmann MW, et al. Live birth after ovarian tissue autotransplantation following overnight transportation before cryopreservation. *Fertil Steril*. 2012;97(2):387–90.
11. Behringer K, Wildt L, Mueller H, Mattle V, Ganitis P, van den Hoonaard B, et al. No protection of the ovarian follicle pool with the use of GnRH-analogues or oral contraceptives in young women treated with escalated BEACOPP for advanced-stage Hodgkin lymphoma. Final results of a phase II trial from the German Hodgkin Study Group. *Ann Oncol Off J Eur Soc Med Oncol ESMO*. 2010;21(10):2052–60.
12. Kim MK, Lee DR, Han JE, Kim YS, Lee WS, Won HJ, et al. Live birth with vitrified-warmed oocytes of a chronic myeloid leukemia patient nine years after allogeneic bone marrow transplantation. *J Assist Reprod Genet*. 2011;28(12):1167–70.
13. Garcia-Velasco JA, Domingo J, Cobo A, Martínez M, Carmona L, Pellicer A. Five years' experience using oocyte vitrification to preserve fertility for medical and nonmedical indications. *Fertil Steril*. 2013;99(7):1994–9.
14. Alvarez M, Solé M, Devesa M, Fábregas R, Boada M, Tur R, et al. Live birth using vitrified-warmed oocytes in invasive ovarian cancer: case report and literature review. *Reprod Biomed Online*. 2014;28(6):663–8.
15. da Motta ELA, Bonavita M, Alegretti JR, Chehin M, Serafini P. Live birth after 6 years of oocyte vitrification in a survivor with breast cancer. *J Assist Reprod Genet*. 2014;31(10):1397–400.
16. Martinez M, Rabadan S, Domingo J, Cobo A, Pellicer A, Garcia-Velasco JA. Obstetric outcome after oocyte vitrification and warming for fertility preservation in women with cancer. *Reprod Biomed Online*. 2014;29(6):722–8.
17. Lawrenz B, Fehm T, von Wolff M, Soekler M, Huebner S, Henes J, et al. Reduced pretreatment ovarian reserve in premenopausal female patients with Hodgkin lymphoma or non-Hodgkin-lymphoma—evaluation by using antimüllerian hormone and retrieved oocytes. *Fertil Steril*. 2012;98(1):141–4.