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ORIGINAL ARTICLE *Reproductive biology*

Similar *in vitro* maturation rates of oocytes retrieved during the follicular or luteal phase offer flexible options for urgent fertility preservation in breast cancer patients

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STUDY QUESTION: Are *in vitro* maturation (IVM) rates of cumulus-oocyte complexes (COCs), retrieved from breast cancer patients seeking urgent fertility preservation (FP) before neoadjuvant chemotherapy, different between those recovered in the follicular or in the luteal phase of the cycle?

SUMMARY ANSWER: The present investigation reveals no major difference in the number of COCs recovered or their IVM rates whatever the phase of the cycle at which egg retrieval is performed, suggesting that IVM is a promising tool for breast cancer patients seeking urgent oocyte cryopreservation.

WHAT IS KNOWN ALREADY: FP now represents a standard of care for young cancer patients having to undergo gonadotoxic treatment. Mature oocyte cryopreservation after IVM of COCs has been proposed for urgent FP, especially in women, who have no time to undergo ovarian stimulation, or when it is contraindicated.

STUDY DESIGN, SIZE, DURATION: From January 2011 to December 2014, we prospectively studied 248 breast cancer patients awaiting neoadjuvant chemotherapy, aged 18–40 years, candidates for oocyte vitrification following IVM, either at the follicular or the luteal phase of the cycle.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Serum anti-Müllerian hormone and progesterone levels and antral follicle count (AFC) were measured prior to oocyte retrieval. Patients were sorted into two groups according to the phase of the cycle during which eggs were harvested (Follicular phase group, $n = 127$ and Luteal phase group, $n = 121$). Number of COCs recovered, maturation rates after 48 h of culture and total number of oocytes cryopreserved were assessed. Moreover, the oocyte retrieval rate (ORR) was calculated by the number of COCs recovered $\times$ 100/AFC.

MAIN RESULTS AND THE ROLE OF CHANCE: In the Follicular and the Luteal phase groups, women were comparable in terms of age, BMI and markers of follicular ovarian status. There was no significant difference in the number of COCs recovered (mean $\pm$ SEM), 9.3 $\pm$ 0.7 versus 11.1 $\pm$ 0.8, and ORR (median (range)) 43.1 (1–100) versus 47.8 (7.7–100)%. Moreover, maturation rates after 48 h of culture (median (range)) were comparable in the follicular and luteal phase groups, 66.7 (20–100) versus 64.5 (0–100)%. Finally, the total number of oocytes cryopreserved (mean $\pm$ SEM) was similar in both groups (6.2 $\pm$ 0.4 versus 6.8 $\pm$ 0.5).

LIMITATIONS, REASONS FOR CAUTION: Despite the intact meiotic competence of immature oocytes recovered during the follicular or the luteal phase, there is a dramatic lack of data regarding the outcome of IVM oocytes cryopreserved in cancer patients.

WIDER IMPLICATIONS OF THE FINDINGS: IVM of oocytes may be an interesting method of FP in urgent situations. Improving the culture conditions will be needed to increase the maturation rates and the overall potential of *in vitro* matured oocytes.

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Introduction

Breast cancer (BC) is the most common malignant tumor in women (Siegel *et al.*, 2013), and accounts for one-third of cancers in those of reproductive age (DeSantis *et al.*, 2011). Many young BC patients have not yet completed their families at the time of diagnosis (Hayat et al., 2007), and are faced with the prospect of ovarian function loss, and therefore infertility, induced by gonadotoxic chemotherapy combined with physiological ovarian aging occurring during the treatment period when pregnancy is precluded (Hickey *et al.*, 2009). Consequently, it is considered that less than 10% of women treated for invasive BC under age 40 have children post-diagnosis (Mueller *et al.*, 2003; Blakely *et al.*, 2004; Cvancarova *et al.*, 2009). The reproductive potential of survivors has become a major concern since 50% of young cancer patients report a desire for pregnancy (Partridge *et al.*, 2004). Over the last decade, the demand for fertility preservation (FP) has dramatically increased and now represents a standard of care for young patients having to undergo gonadotoxic cancer treatment (ISFP Practice Committee *et al.*, 2012; Loren *et al.*, 2013).

Several strategies for FP have been developed, by applying recent cryotechnologies and more established reproductive techniques. Currently, cryopreservation of oocytes and/or embryos after controlled ovarian hyperstimulation represents the most established methods for preserving female fertility (Loren *et al.*, 2013). However, the risk of supraphysiologic serum estradiol levels reached after ovarian stimulation should be considered in hormone-sensitive tumors such as BC, even when the FP procedures are performed after surgical removal of the tumor and before adjuvant chemotherapy. In addition, a growing number of young BC patients are candidates for urgent neoadjuvant chemotherapy, preceding surgery (Kaufmann *et al.*, 2006). Although this strategy may be interesting from an oncologic standpoint, it significantly complicates FP attempts, since the window for optimal preservation between diagnosis and initiation of gonadotoxic treatment is dramatically narrowed, and the tumor is still in place during follicle stimulation.

Recently, retrieval of cumulus-oocyte complexes (COCs) from small antral follicles, without exogenous FSH administration, has been proposed as an option for young patients seeking FP, in particular when controlled ovarian hyperstimulation is unfeasible or unsuitable (Berwanger *et al.*, 2012). Indeed, after *in vitro* maturation (IVM), the metaphase II oocytes may be frozen or fertilized for embryo cryopreservation. Although the clinical efficiency of this procedure remains hard to assess at present, it may constitute the safer option for patients wishing oocyte cryopreservation before breast tumorectomy. In addition, IVM can be combined with ovarian tissue cryopreservation to cumulate FP strategies (Berwanger *et al.*, 2012). However, whether the potential of COCs to mature *in vitro* is comparable according to the phase of the cycle at which immature eggs are recovered in BC patients remains unknown.

Materials and Methods

Subjects

From 2011 to 2014, two-hundred forty-eight BC patients, 18–40 years of age, candidates for urgent FP using IVM before neoadjuvant chemotherapy were prospectively studied (Fig. 1). Patients in both groups met the following inclusion criteria: (i) diagnosis of invasive BC; (ii) indication of neoadjuvant chemotherapy; (iii) no current hormone therapy; (iv) no previous history of chemotherapy; (v) presence of two ovaries; (vi) adequate visualization of ovaries at transvaginal ultrasound scans; (vii) total number of small antral follicles (3–10 mm in diameter) >10 follicles, including both ovaries. Single patients were offered oocyte cryopreservation but if they were engaged they had the possibility of choosing oocyte or embryo freezing.

The study was approved by our Institutional Review Board (IRB) and a written informed consent was obtained from each patient or couple.

Hormonal measurements and ultrasound scans

Before oncofertility counseling, each woman underwent a blood sampling by venipuncture for measurement of serum anti-Müllerian hormone (AMH) and progesterone levels and a transvaginal ovarian ultrasound scan for antral follicle assessment.

Serum AMH levels were determined using an ultrasensitive enzyme-linked immunosorbent assay (ELISA) (Beckman-Coulter, Villepinte, France). Intra- and inter-assay coefficients of variation were fewer than 6% and fewer than 10%, respectively; lower detection limit was 0.13 ng/ml, and linearity was up to 21 ng/ml. Serum progesterone levels were determined by an automated multi-analysis system using a chemiluminescence technique (Advia-Centaur; Bayer Diagnostics, Puteaux, France). For progesterone, the lower detection limit was 0.1 ng/ml; linearity was up to 60 ng/ml, and intra- and inter-assay coefficients of variation were 8 and 9%, respectively.

Ultrasound scans were performed using a 3.7–9.3 MHz multi-frequency transvaginal probe (RIC5-9H, Voluson 730 Expert, General Electric Medical Systems, Paris, France) by two operators, who were blinded to the results of hormone assays. The objective of ultrasound examination was to evaluate the number and sizes of small antral follicles. All follicles measuring 3–20 mm in mean diameter (mean of two orthogonal diameters) in each ovary were considered. To optimize the reliability of ovarian follicular assessment, the ultrasound scanner used was equipped with a tissue harmonic imaging system (Thomas and Rubin, 1998), which allowed improved image resolution and adequate recognition of follicular borders. Intra-analysis CV for follicular and ovarian measurements were <5% and their lower limit of detection was 0.1 mm.

Luteal phase was defined by the presence of a corpus luteum and a serum progesterone level >3 ng/ml.

Technique

Oocyte retrieval was performed, 36 h after administration of hCG (Gonadotrophine Chorionique Endo, Organon Pharmaceutique, Saint-Denis,

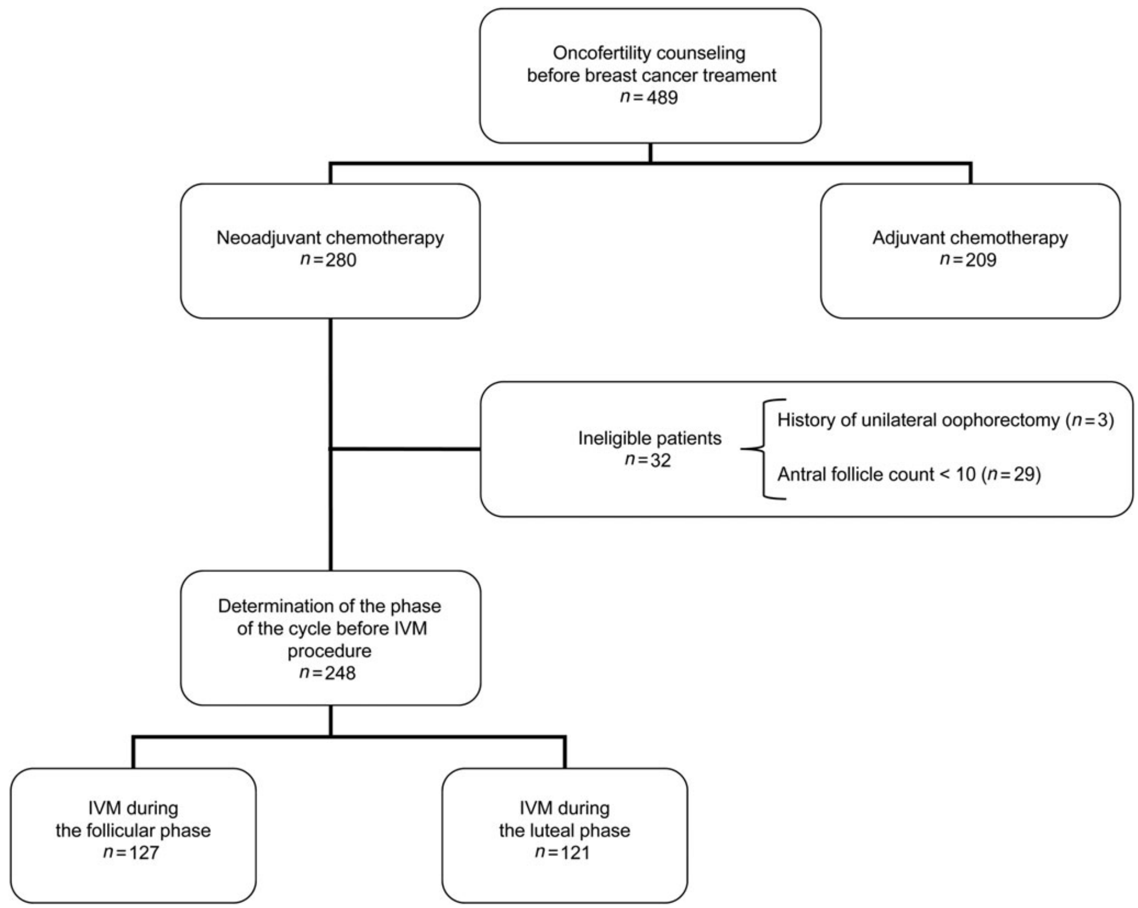

Figure 1 Flow chart for patient recruitment to the study. IVM, *in vitro* maturation.

France), under moderate sedation, using a 19-Gauge needle (K-OPS-7035-Wood; Cook, France) guided by vaginal ultrasound. Aspiration pressure was fixed at 7.5 kPa. Follicular fluid containing COCs was aspirated into pre-heated 15 ml Nucleon™ tubes (Nunc A/S, Denmark), filled with 3 ml of sodium heparinatum 2 UI/ml (Sanofi-Synthelabo, France). Follicular fluid was then analyzed in Nucleon™ culture dishes (Nunc A/S, Denmark), where COCs were isolated and washed with a culture medium, Universal IVF Medium® (Origio, Denmark). COCs were then placed into a culture dish (Becton Dickinson, USA) containing 1 ml of culture medium IVM® (Medi Cult, Danemark) enriched with 20% inactivated maternal serum, 0.75 UI/ml, FSH and 0.75 UI/ml of LH Menopur® (Ferring, Germany) (Chian *et al.*, 2002). COCs were then incubated at 37°C in a 5% CO_2/20% O_2/N_2 gas mixture. After 24 h of culture, all COCs were denuded with a hyaluronidase solution (SynVitro Hyadase, Origio, Denmark) and nuclear oocyte maturation was assessed. Depending on the patient's choice mature metaphase II oocytes with extruded polar body were frozen on the same day or fertilized by ICSI and zygotes were frozen 24 h later.

Oocytes having failed to mature after 24 h were kept for an additional 24 h of culture. After 48 h, oocytes that reached metaphase II stage were frozen, while immature eggs were discarded.

Oocyte retrieval rate

To objectively assess the efficiency of immature oocyte retrieval in follicular and luteal phase groups, we decided to analyze the oocyte retrieval rate (ORR), calculated as the ratio of the number of COCs recovered $\times$ 100/antral follicle count.

Oocyte/zygote cryopreservation

Since vitrification was unauthorized in France before July 2011, all mature oocytes obtained before this date were cryopreserved using slow-freezing method (Fabbri *et al.*, 1998). From January 2012 onwards, oocytes or embryos were vitrified (Kuwayama *et al.*, 2005). Briefly, for slow freezing, oocyte freezing solution containing 1,2-propandiol (1,2-PROH) and sucrose as cryoprotectants was used according to the manufacturer recommendations (OocyteFreeze, Origio, Denmark). After washing in solution 1 (phosphate-buffered saline solution), oocytes were first incubated 10 min at room temperature in the freezing solution 1 (1.5M 1,2-PROH), and then transferred into the freezing solution 2 (1.5M 1,2-PROH+0.3M sucrose). At most two oocytes were loaded into one 0.3 ml high security straw (CBS, Cryobiosystem, France) and cooled into an automated controlled-rate freezer (Planer, Products Limited, Sunbury, UK) with a manual seeding performed at −8°C. After cooling, straws with oocytes were stored in liquid nitrogen.

All zygotes were frozen using a vitrification procedure. Vitrification was performed using the closed Rapid-i vitrification system (Vitrolife, France) and Blast-freeze media (Vitrolife) as recommended by Vitrolife for oocyte or zygote cryopreservation. The entire procedure was performed at 37°C according to the manufacturer's instructions (cryoprotectant concentrations are not detailed in the commercial kits). Oocytes or zygotes were first incubated in the Vitri 1-blast solution (containing no cryoprotectant) for 5–20 min, then placed in the Vitri 2 solution (containing ethylene-glycol) during 2 min and finally in the Vitri 3 solution (containing ethylene-glycol, 1,2-PROH and sucrose) for exactly 45 s. During this time, at most 2 oocytes or zygotes were loaded in one Rapid-i device and placed in high security straws previously cooled in liquid nitrogen. Straws are then sealed and immersed in liquid nitrogen before storage.

Ovarian tissue cryopreservation

In women who chose a double strategy of FP combining ovarian tissue cryopreservation and IVM, laparoscopy was performed after oocyte retrieval. A piece of the ovary containing the highest number of antral follicles on ultrasound scan was removed using scissors. The sample was immerged in a sterile dish with Ferticult Hepes (JCD, France) at 4°C on ice. Fragments of 5 × 10 mm were obtained after dissection of the tissue with scissors and scalpel and transferred into cryotubes containing 800 μl of Ferticult HEPES medium on ice. Medium was then removed and replaced twice by 800 μl of Ferticult Hepes medium added with 10% v/v of dimethyl sulfoxide as cryoprotectant and 2% v/v of patient's inactivated serum. Cryotubes containing one or two ovarian fragments were cooled into an automated controlled-rate freezer (Planer, Products Limited, Sunbury, UK), following the methodology described by Donnez *et al.* (2006).

Statistical analysis

The measures of central tendency and variability used were the mean ± SEM when data distribution was normal, and the median and the ranges when normality could not be ascertained. Patients were ranked in the 'Follicular group' when the egg retrieval was performed during their follicular phase or in the 'Luteal group' when egg retrieval occurred during their luteal phase. Differences between continuous variables from these two groups were evaluated with Student's t or Mann–Whitney tests when appropriate. Categorical variables in the two groups were compared using the two-sided Pearson χ^2 test. A *P*-value <0.05 was considered statistically significant.

Results

Patients' and hormone-follicle characteristics

Overall, the mean age of patients was 31.5 ± 0.3 years (Table I). All had a diagnosis of invasive ductal carcinoma and 35 patients (18%) presented node invasion. Breast tumor expressed estrogen and/or progesterone receptors in 150 patients. The strategy of neoadjuvant chemotherapy was decided by the oncologist, after multidisciplinary discussion of each patient's chart. Analysis of the follicular ovarian status revealed mean antral follicle count and serum AMH levels at 22.1 ± 0.8 follicles and 4.75 ± 0.33 ng/ml, respectively.

After oncofertility counseling, a large majority of patients decided to freeze oocytes ($n = 213$) instead of embryos ($n = 35$). In addition, 34 patients chose ovarian tissue cryopreservation in association with oocyte cryopreservation.

Comparison of IVM results according to the phase of the cycle during which egg retrieval was performed

Immature oocyte retrieval was performed during follicular or luteal phase in 127 and 121 patients, respectively (Table II). In the follicular group, 43 (33.8%) patients had a dominant follicle on the day of hCG. The overall ORR was 41.4%, and did not differ significantly whatever the phase of egg retrieval (43.1 (1–100) versus 47.8 (7.7–100), in the Follicular and Luteal phase groups, respectively, *NS*). In addition, the number of COCs recovered was comparable in both groups (9.3 ± 0.7 versus 11.1 ± 0.8, respectively, *NS*). With similar maturation rates after 24 and 48 h of culture (59.0 ± 24.2% versus 54.7 ± 24.0%, and

Table I Patients' characteristics.

Age (years)	31.5 ± 0.3
BMI (kg/m^2)	22.2 ± 0.3
Smokers	83 (33%)
Gravidity	0.7 ± 0.1
Parity	0.4 ± 0.1
Menstrual cycles	
Regular	198 (79.8)
Irregular	50 (20.2)
Hormonal status of the tumor	
Positive hormonal receptors	150 (60.5)
Negative hormonal receptors	98 (39.5)
Antral follicle count	22.1 ± 0.8
Serum anti-Müllerian hormone levels (ng/ml)	4.75 ± 0.33
Immature oocyte retrieval during follicular phase	127 (51.2)
Immature oocyte retrieval during luteal phase	121 (48.8)
No. of cycles with presence of a dominant follicle	43 (17.3)

Data are mean ± SEM or *n* (%).

Table II Comparison of *in vitro* maturation results according to the phase of the cycle during which egg retrieval was performed.

	Follicular phase group ($n = 127$)	Luteal phase group ($n = 121$)	*P*
Age (years)	31.9 ± 0.4	31.0 ± 0.4	NS
BMI (kg/m^2)	22.2 ± 0.4	22.1 ± 0.5	NS
Antral follicle count	21.4 ± 1.0	22.9 ± 1.2	NS
Serum anti-Müllerian hormone levels (ng/ml)	4.44 ± 0.4	5.03 ± 0.5	NS
Serum progesterone levels on the day of hCG (ng/ml)	0.2 ± 0.1	6.7 ± 0.2	*<0.0001*
No. of cumulus oocyte complexes recovered	9.3 ± 0.7	11.1 ± 0.8	NS
Oocyte recovery rate (%)[a]	43.1 (1–100)	47.8 (7.7–100)	NS
No. of *in vitro* matured oocytes	5.9 ± 0.4	6.8 ± 0.5	NS
Maturation rate (%)	66.7 (20–100)	64.5 (0–100)	NS
No. of oocytes cryopreserved	6.2 ± 0.4	6.8 ± 0.5	NS

Data are mean ± SEM or median (range).
NS, not significant ($P > 0.05$).
[a]No. of COCs recovered × 100/Antral follicle count.

9.0 ± 14.3% versus 9.0 ± 14.3%, respectively, *NS*), the number of *in vitro* matured oocytes was comparable (5.9 ± 0.4 versus 6.8 ± 0.5 oocytes, respectively). In the Follicular phase group the number of oocytes available for cryopreservation was increased by the mature eggs recovered from the dominant follicle, which are not included in the results.

Interestingly, the capacity of IVM metaphase two oocytes to be fertilized was similar whatever the phase of the cycle at which the COCs were recovered (66.3 ± 30.7 versus 62.7 ± 28.0%, respectively), leading to a comparable number of zygotes cryopreserved (78.7 ± 2.8 versus 78.4 ± 2.0 zygotes, respectively).

No complication was reported after the procedure in either group and all patients were able to start chemotherapy without delay.

Discussion

Our study investigated whether, in BC patients, the potential of COCs for IVM is comparable whatever the phase of the cycle at which immature eggs are recovered. In these patients, FP procedures are classically performed in the 2–6 weeks interval between surgical removal of the tumor and initiation of adjuvant chemotherapy. This time-frame allows oocyte or embryo cryopreservation after controlled ovarian hyperstimulation, which is the most established method for FP (ISFP *et al.*, 2012). However, an increasing proportion of young women are included in protocols using neoadjuvant therapy, administered before surgery. This strategy is often proposed in women having clinically positive nodes or a ≥2 cm tumor, as these women are likely to harbor micrometastases (Kaufmann *et al.*, 2006). Unfortunately, use of neoadjuvant chemotherapy further complicates FP strategy for at least two major reasons. First, the window for optimal preservation between diagnosis and initiation of gonadotoxic treatment is dramatically reduced. Second, the persistence of the tumor within the breast accounts for theoretical risk of tumoral expansion if controlled ovarian hyperstimulation is performed, even with protocols combining exogenous FSH administration and aromatase inhibitors to reduce serum estradiol levels (Ström *et al.*, 2004). Consequently, candidates for neoadjuvant chemotherapy are usually offered ovarian tissue cryopreservation (Kim *et al.*, 2011a). However, this technique is considered experimental, with few live births reported (Donnez *et al.*, 2013; Loren *et al.*, 2013). In addition, the surgical procedure for ovarian tissue cryopreservation is sometimes considered too invasive by women having just been diagnosed with cancer.

Recently immature oocyte retrieval in an unstimulated menstrual cycle has been proposed as an attractive option for preserving female fertility, since it allows oocyte and/or embryo cryopreservation without exogenous FSH administration (Berwanger *et al.*, 2012). Some authors have reported that oocyte maturation rate per collected oocyte was significantly higher for oocytes matured before vitrification when compared with those obtained with oocytes vitrified before IVM (Fasano *et al.*, 2012). For this reason, our policy rests on the cryopreservation of IVM oocytes. The present investigation shows, on a large series of BC patients, that IVM is a safe and feasible technique for attempting to preserve female fertility in emergency, before gonadotoxic treatment. Indeed, the fact that immature oocytes can be retrieved at any time in the menstrual cycle, with similar results in terms of output and maturation rates, allows this technique to be considered as an option for urgent FP. In addition, since IVM does not require ovarian stimulation, it might be considered (in combination with ovarian tissue cryopreservation?) in BC patients having to undergo neoadjuvant chemotherapy.

Our results showed that egg retrieval during the late follicular phase tended to lead to a higher number of mature oocytes cryopreserved. This is related to the recovery of a mature oocyte from the dominant follicle. However, Son *et al.*, previously showed that optimal results of IVM cycles were obtained when the diameter of the dominant follicles was less than 14 mm (Son *et al.*, 2008).

Finally, a mean of 6.4 ± 0.3 oocytes were frozen, which is comparable to results reported by Azim *et al.*, using protocols of ovarian stimulation with letrozole in BC patients (Azim *et al.*, 2008). It is however possible that oocytes cryopreserved after controlled ovarian hyperstimulation may harbor better developmental potential as compared with eggs matured *in vitro*. Indeed, many studies performed in infertile women have reported better pregnancy and live birth rates after ovarian stimulation when compared with IVM (Child *et al.*, 2002; Gremeau *et al.*, 2012; Fadini *et al.*, 2013).

The central finding of our study is the comparable results of IVM regardless of the phase of the menstrual cycle at which immature oocytes collection was performed. Indeed, the total number of COCs recovered, as well as the ORR were similar during follicular and luteal phases. ORR in cancer patients was also comparable to rates reported in PCOS patients (Fadini *et al.*, 2013). This innovative tool was calculated by dividing the number of immature oocytes retrieved by the number of small antral follicles observed before egg collection. Since the ORR considers the amount of small antral follicles, as opposed to the absolute number of immature oocytes obtained, it is probably a more accurate way to assess objectively the efficiency of the retrieval procedure.

Several lines of evidence indicate the existence of multiple major follicle recruitment waves during a normal menstrual cycle. Indeed, Baerwald *et al.,* showed up to three waves of follicular recruitment in healthy volunteers having undergone daily ultrasonographic and hormonal evaluation (Baerwald *et al.*, 2003). This physiologic data may explain the comparable ORR during the follicular or the luteal phase.

The competence of COCs retrieved during the luteal phase could be questioned. However, some studies showed that immature oocytes retrieved during Caesarean section (with exposure to high serum progesterone concentrations) are capable of IVM and could lead to live births after fertilization (Chian *et al.*, 2002, 2009a; Rao *et al.*, 2004). More recently, Oktay *et al.,* reported immature oocyte collection in the luteal phase as a rescue option for a patient who experiences a premature LH surge during ovarian stimulation for FP (Oktay *et al.*, 2008). Of the four COCs recovered, 50% reached maturation and were vitrified. Furthermore, Demirtas *et al.*, showed, in three oncofertility patients, that immature oocytes retrieved during the luteal phase of an unstimulated cycle could reach metaphase II (Demirtas *et al.*, 2008). The only available comparative study of IVM results in FP candidates having undergone immature oocyte retrieval during either the follicular or luteal phase was recently published but included a very small population (Maman *et al.*, 2011). Eighteen cancer patients underwent IVM, five in their luteal phase and 13 in their follicular phase. The authors failed to find any significant difference in the number of COCs recovered, maturation rates, fertilization rates, or the total number of oocytes and embryos that were cryopreserved (Maman *et al.*, 2011). We report, to our knowledge, the largest series of IVM for FP in BC patients. Overall, our results are similar to those obtained by Maman *et al.*, and confirm the intact meiotic competence of immature oocytes recovered during the luteal phase, as well as their capacity to reach zygote stage after fertilization. These data are contradictory with animal studies, having reported a superiority of immature oocytes harvested during the early follicular phase when compared with those obtained during the late follicular or the luteal phase (Chian, 2004). Moreover, we should be aware that the real potential (i.e. to lead to live births) of frozen IVM oocytes collected in different phases of the menstrual cycle is still undetermined.

However, there is a dramatic lack of data regarding the outcome of oocytes cryopreserved in cancer patients. Indeed, whatever the technique used (i.e. ovarian stimulation or IVM) and the timing of either the initiation of exogenous gonadotrophin administration or immature oocyte retrieval (i.e. follicular or luteal phase), very few live births have been reported in these women (Porcu *et al.*, 2008; Kim *et al.*, 2011b; Garcia-Velasco *et al.*, 2013; Prasath *et al.*, 2014). As a consequence, caution should be taken before concluding to the same efficiency of IVM performed during the follicular or the luteal phase.

Although ORR was quite good in our patients, immature oocyte retrieval is often unpredictable and could be very disappointing in some patients. Therefore, patients should be objectively informed of this limit, which could lead to a small number of mature oocytes or embryos cryopreserved in the end. Consequently, a combination of immature oocyte retrieval and ovarian tissue cryopreservation should always be considered, in order to cumulate the FP strategies.

At present, only four live births have been reported after oocyte vitrification following IVM in healthy patients (Chian *et al.*, 2009b). In addition one successful live birth has recently been published after embryo vitrification following IVM in a cancer patient (Zhang *et al.*, 2010).

Previous studies showed that embryos obtained from IVM oocytes displayed increased aneuploidy rates (Zhang *et al.*, 2010) and lower implantation potential (Clyde *et al.*, 2003; Magli *et al.*, 2006; Zhang *et al.*, 2010; Yakut *et al.*, 2012) when compared with those obtained from oocytes matured *in vivo*. However, it is important to keep in mind that these results were described in PCOS patients, which is not an optimal model since their oocytes may be altered by the ovarian disease itself (Söderström-Anttila *et al.*, 2005). In addition, the decreased implantation rates of fresh embryos obtained after IVM could also be explained by suboptimal endometrial priming. A recent data has shown that an all freeze strategy could improve the success rates of IVM (De Vos *et al.*, 2011). Given these data, it is possible that cancer patients having oocytes frozen could expect better pregnancy rates.

The present investigation shows that the retrieval of immature oocytes from small antral follicles as well as the IVM rates remain similar whatever the period of the menstrual cycle. In the end, the number of mature oocytes cryopreserved were comparable. These data suggest that IVM can be offered to BC patients seeking urgent FP.

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Authors' roles

M.G. contributed to the design of the study and participated in the analysis and interpretation of data, and in drafting the manuscript or revising it critically for important intellectual content. M.P. participated in the collection, analysis and interpretation of the data, and contributed to writing the manuscript. S.L.P. participated in the collection of the data and contributed to the critical revision of the manuscript. C.S. participated in the analysis and interpretation of data, and contributed to the critical revision of the manuscript. R.F. contributed to the design of the study and to the critical revision of the manuscript. N.F. contributed to the design of the study and to the critical revision of the manuscript.

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Conflict of interest

None declared.

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