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ORIGINAL ARTICLE *Fertility control*

Emergency IVF for embryo freezing to preserve female fertility: a French multicentre cohort study

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STUDY QUESTION: What are the outcomes of French emergency IVF procedures involving embryo freezing for fertility preservation before gonadotoxic treatment?

SUMMARY ANSWER: Pregnancy rates after emergency IVF, cryopreservation of embryos, storage, thawing and embryo transfer (embryo transfer), in the specific context of the preservation of female fertility, seem to be similar to those reported for infertile couples undergoing ART.

STUDY DESIGN, SIZE, DURATION: A French retrospective multicentre cohort study initiated by the GRECOT network—the French Study Group for Ovarian and Testicular Cryopreservation. We sent an e-mail survey to the 97 French centres performing the assisted reproduction technique in 2011, asking whether the centre performed emergency IVF and requesting information about the patients' characteristics, indications, IVF cycles and laboratory and follow-up data. The response rate was 53.6% (52/97).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Fourteen French centres reported that they performed emergency IVF (56 cycles in total) before gonadotoxic treatment, between 1999 and July 2011, in 52 patients.

MAIN RESULTS AND THE ROLE OF CHANCE: The patients had a mean age of 28.9 ± 4.3 years, and a median length of relationship of 3 years (1 month–15 years). Emergency IVF was indicated for haematological cancer (42%), brain tumour (23%), sarcoma (3.8%), mesothelioma ($n = 1$) and bowel cancer ($n = 1$). Gynaecological problems accounted for 17% of indications. In 7.7% of cases, emergency IVF was performed for autoimmune diseases. Among the 52 patients concerned, 28% ($n = 14$) had undergone previous courses of chemotherapy before beginning controlled ovarian stimulation (COS). The initiation of gonadotoxic treatment had to be delayed in 34% of the patients ($n = 19$). In total, 56 cycles were initiated. The mean duration of stimulation was 11.2 ± 2.5 days, with a mean peak estradiol concentration on the day on which ovulation was triggered of 1640 ± 1028 pg/ml. Three cycles were cancelled due to ovarian hyperstimulation syndrome ($n = 1$), poor response ($n = 1$) and treatment error ($n = 1$). A mean of 8.2 ± 4.8 oocytes were retrieved, with 6.1 ± 4.2 mature oocytes and 4.4 ± 3.3 pronuclear-stage

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embryos per cycle. The mean number of embryos frozen per cycle was 4.2 ± 3.1. During follow-up, three patients died from the consequences of their disease. For the 49 surviving patients, 22.5% of the couples concerned ($n = 11$) requested embryo replacement. A total of 33 embryos were thawed with a post-thawing survival rate of 76%. Embryo replacement was finally performed for 10 couples with a total of 25 embryos transferred, leading to one biochemical pregnancy, one miscarriage and three live births. Clinical pregnancy rate and live birth per couple who wanted a pregnancy after cancer were, respectively, 36% (95% CI = 10.9–69.2%) and 27% (95% CI = 6.0–61%).

LIMITATIONS, REASONS FOR CAUTION: The overall response rate for clinics was 53.6%. Therefore, it is not only that patients may not have been included, but also that those that were included were biased towards the University sector with a response rate of 83% (25/30) for a small number of patients.

WIDER IMPLICATIONS OF THE FINDINGS: According to literature, malignant disease is a risk factor for a poor response to COS. However, patients having emergency IVF before gonadotoxic treatment have a reasonable chance of pregnancy after embryo replacement. Embryo freezing is a valuable approach that should be included among the strategies used to preserve fertility.

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Key words: cancer / premature ovarian failure / fertility preservation / IVF / embryo freezing

Introduction

With improvements in cancer treatment, it has become essential to preserve female fertility before the administration of gonadotoxic treatment and to provide patients with appropriate information (Woodruff, 2010). The toxicity to the gonads of anticancer treatments was long considered of secondary importance, given the very poor prognosis of affected patients. The question of subsequent fertility has only really become an issue with improvements to patient survival, often due to treatment intensification (Byrne *et al.*, 1992; Bath *et al.*, 2002; Kim, 2006). Improvement of survival rates after certain types of cancer (Belot *et al.*, 2008), and particularly breast cancer (Shigematsu *et al.*, 2011) with a survival rate of 74% at 10 years (Jooste *et al.*, 2013), chronic lymphoid leukaemia (Maynadié *et al.*, 2013) and Hodgkin lymphoma with survival rates exceeding 80% (Gatta *et al.*, 2009), has led to increased consideration of quality of life after cancer. The preservation of female fertility is thus a question that oncologists and reproduction specialists need to address (Niemasik *et al.*, 2012).

There are many indications for fertility preservation, including the most gonadotoxic treatments, such as chemotherapy with alkylating agents, the myeloablative treatments administered before bone marrow transplantation or haematopoietic stem cell transplantation and high-dose abdominal/pelvic radiotherapy (Sonmezer and Oktay, 2004). The strategy proposed for any particular patient should be chosen according to the patient's age, relationship status, desire to have children, the nature of the disease, the type of treatment and the urgency with which cancer treatment needs to be initiated.

Several techniques for preserving female fertility have been developed (Kim *et al.*, 2012): the freezing of ovarian tissue (Donnez *et al.*, 2011), the freezing of mature oocytes (Cobo *et al.*, 2008; Grifo and Noyes, 2010), oocyte maturation *in vitro* (Cao and Chian, 2009) and emergency IVF for embryo freezing (Dolmans *et al.*, 2005).

Few long-term follow-up data are currently available for women undergoing fertility preservation procedures. Oocyte vitrification has only recently been authorised in France (July 2011). In the context of oocyte donation, pregnancy rates after IVF have been shown to be identical for vitrified and fresh oocytes (Cobo *et al.*, 2010). However, as far as we are aware, no follow-up data are available for women undergoing oocyte vitrification in the particular context of fertility preservation before gonadotoxic treatment. The use of IVF and embryo freezing is well established for the treatment of infertile couples and has been used for 25 years (Zeilmaker *et al.*, 1984), such that long-term data are abundant (Wennerholm *et al.*, 2009). Consequently, emergency IVF for embryo freezing is believed to give the highest pregnancy rates in the context of fertility preservation (Levine *et al.*, 2010).

However, in the particular context of IVF for malignant disease, little is known about long-term clinical outcomes (Friedler *et al.*, 2012).

The objective of our study was to provide an overview of fertility preservation practices in an oncological context in France, with the assessment of the indications, feasibility and hopes of pregnancy after emergency IVF performed before gonadotoxic treatment.

Materials and Methods

We carried out a retrospective multicentre study, in which an e-mail survey was sent to all French ART centres ($n = 97$), requesting information about their emergency IVF practices. Each centre was sent two files (questionnaire available as Supplementary material). The first contained a questionnaire asking whether the centre performed emergency IVF before gonadotoxic treatment. If not, they were asked why: no requests from oncologists, no indication or a contraindication for controlled ovarian stimulation (COS), ethical reasons for not freezing embryos in this context. The second file contained a questionnaire to be completed by ART centres that performed emergency IVF before gonadotoxic treatment. This questionnaire aimed to assess the patients' characteristics, diseases, IVF cycles and follow-up after embryo freezing. No information that might allow individual patients to be identified was requested, ensuring the anonymity of all respondents and patients. All data were collected through a review of medical files.

Outcome data

The data collected for patient characteristics were: age, parity, length of relationship, length of time for which the couple had been planning to have children, indication for fertility preservation, taking the prognosis for long-term survival into account when selecting the fertility preservation strategy, history of chemotherapy before IVF and the need to delay gonadotoxic treatment. Where possible, ovarian reserves were evaluated by the determination of baseline serum concentrations of FSH, estradiol (E_2) and anti-Mullerian

hormone (AMH) on the third day of the cycle and antral follicular count on vaginal ultrasound examination. Information was requested concerning the type of COS protocol used, with the total dose of gonadotrophin needed, the duration of stimulation and E_2 levels on the day on which ovulation was triggered. Laboratory data recorded the numbers of retrieved oocytes, metaphase II oocytes, 2PN (pronuclear) zygotes and fertilization rate, embryo stage on the day of freezing and the total number of embryos frozen per cycle. The clinical variables recorded for the follow-up of each patient included requests for embryo replacement and pregnancy outcome. Pregnancy was defined as positive hCG detection 2 weeks after embryo transfer, and clinical pregnancy was defined as the presence of a gestational sac at the first ultrasound scan, at 6 weeks of gestation.

Statistical analysis

All data were analysed with EXCEL software (Microsoft Corp., Redmond, WA, USA). Descriptive data are expressed as means ± standard error and percentages. Some results are also expressed as medians and ranges.

Results

Uneven access to fertility preservation techniques

The response rate was 53.6%, with 52 ART centres completing the survey. The response rate for university teaching hospitals in France was higher, at 83% ($n = 25/30$). In total, 37 of the 52 ART centres reported that they did not perform emergency IVF and embryo freezing before gonadotoxic treatment because they had never received a specific request for this procedure from an oncologist working in their city ($n = 27$) or because the indication was inappropriate ($n = 9$); 1 of these 37 centres did not perform embryo freezing in this context for ethical reasons. Only 1 of the 27 private ART centres had performed emergency IVF. Fourteen ART centres performed emergency IVF and embryo freezing before gonadotoxic treatment between 1999 and July 2011, on 52 patients (56 IVF cycles).

Characteristics of the patients

The patients had a mean age of 28.9 ± 4.3 years and 92% of them were nulliparous ($n = 48$). All of the patients were living in a couple, with a median relationship length of 3 years (1 month–15 years). In 58% of these couples, no plans had been made to have children before the diagnosis of the disease. The characteristics of the patients and the type of disease leading to emergency IVF are summarized in Table I. Baseline serum concentrations of FSH and E_2 on the third day of the cycle were available for 13 patients, AMH concentrations were available for 17 patients and antral follicular counts obtained from ultrasound scans were available for 22 patients.

Description of the IVF cycles

We found that 28% of the 52 patients had undergone a previous course of chemotherapy ($n = 14$) before beginning COS for IVF. Multidisciplinary case discussions resulted in the postponing of gonadotoxic treatment in 34% of patients ($n = 19$). The decision to carry out emergency IVF to preserve fertility took into account the prognosis for survival and the chances of remission in 50% of the cases ($n = 26$), but not in the other 50%. Another method of fertility preservation (cryopreservation of ovarian tissue) was also done in 10% of cases ($n = 5$).

Table I: Emergency IVF: characteristics of the patients and their underlying malignancies (subgroups of cancers in bold)

Age (years)	28.9 ± 4.3 (21–40)
Length of relationship (months)	46.4 ± 35
Nulliparous	48 (92%)
Antral follicular count	14.3 ± 8.8
FSH (third day of cycle; IU/l)	6.4 ± 3
E_2 (third day of cycle; pg/ml)	39.6 ± 21.1
AMH (ng/ml)	2.7 ± 2.5
Underlying malignancy	**52** patients (with cancer)
Haematological	**24 (42%)**
Acute leukaemia	7
Hodgkin lymphoma	7
Non-Hodgkin lymphoma	6
Multiple myeloma	2
Myelodysplasia	1
Thymoma	1
Brain tumours	**11 (23%)**
Glioma	9
Oligoastrocytoma	1
Other	1
Gynaecological	**9 (17%)**
Recurrence of ovarian borderline tumour	5
Breast cancer	4
Sarcoma	**2 (3.8%)**
Synovialosarcoma	1
Breast sarcoma	1
Other cancers	**2 (3.8%)**
Colorectal adenocarcinoma	1
Mesothelioma	1
Systemic autoimmune diseases	**4 (7.7%)**

Data are mean ± SEM (range) or number (%) $n = 52$.

In total, 56 cycles were initiated. COS was performed by an antagonist protocol in 42.8% of cases ($n = 24$), a long-acting GnRH agonist protocol in 30.3% of cases ($n = 17$), a short protocol in 23.2% of cases ($n = 13$) and an aromatase inhibitor protocol in 3.5% of cases ($n = 2$). The mean duration of stimulation was 11.2 ± 2.5 days, with a mean peak E_2 concentration on the day on which ovulation was triggered of 1640 ± 1028 pg/ml. Three cycles were cancelled, due to ovarian hyperstimulation syndrome (OHSS; $n = 1$), an absence of follicular response to COS ($n = 1$) and treatment error ($n = 1$). The laboratory parameters of the other 53 cycles are summarized in Table II.

Outcome

The mean duration of follow-up was 3.3 ± 2.5 years (1–12). Three patients died during the follow-up period, due to the consequences of their disease. Embryo replacement was requested by 22.5% ($n = 11$) of the couples corresponding to the 49 surviving patients. For these 11 couples, a total of 81 mature oocytes were retrieved leading to 48

frozen embryos (Table III); 33 of these embryos were thawed with a post-thawing survival rate of 76%. Embryo transfer was finally performed for 10 couples, 2 of whom had two replacement cycles making a total of 12 embryo transfers: a total of 25 embryos were transferred, leading to one biochemical pregnancy and four clinical pregnancies, resulting in one miscarriage and three live births. Finally, the clinical pregnancy rate and live birth per couple who had an embryo transfer were, respectively, 40% (95% CI = 12.2–73.8%) and 30% (95% CI = 6.7–65.3%) and were 36% (95% CI = 10.9–69.2%) and 27% (95% CI = 6.0–61%) on an intention to treat basis (11 couples), i.e. per couple which wanted a pregnancy after cancer. One couple requested the destruction of their frozen embryos after achieving a spontaneous pregnancy resulting in a live birth. Three couples, in which the woman was treated for haemopathy, achieved spontaneous pregnancies and one couple, in which embryo replacement failed, subsequently achieved pregnancy with oocyte donation.

Table II: Laboratory data after COS for fertility preservation (n = 53)

Total number of oocytes retrieved	8.2 ± 4.8 (2–21)
Metaphase II oocytes	6.1 ± 4.2 (1–20)
2PN zygotes	4.4 ± 3.3 (0–17)
Fertilisation rate (%)	72.3
Total number of embryos frozen per cycle	4.2 ± 3.1 (0–15)
Stage at freezing (n)	
2PN zygote	30
Embryo day 2 or 3	19
Blastocyst	1

Three cycles were cancelled. Unless otherwise stated, results are expressed as means ± SEM (range).

Discussion

We report here the largest cohort to date of patients undergoing emergency IVF in the particular context of fertility preservation in women with malignant disease. The live birth rate for couples undergoing thawed embryo transfer after cancer remission seems to be similar to that for the general population of ART patients. In 2009, 16 838 thawed embryos were transferred into patients for infertility treatment in France with a clinical pregnancy rate per embryo transfer of 18.1% (n = 3052) and a live birth rate of 14.3% (n = 2416; Agence de la Biomédecine, 2010). Unfortunately, the methodology of our retrospective multicentre study made it difficult to match our patients with suitable controls for a case–control study. It would be extremely difficult to constitute an appropriate control group because we could never get enough

Table III: Clinical and laboratory data for frozen-thawed embryo transfers after emergency IVF for fertility preservation

Case	Initial pathology	Age at IVF	Total number of mature oocytes retrieved	Total number of cryopreserved early cleavage embryos	Survival rate after thawing	Total number of thawed transferred embryos	Pregnancy	Outcome
1	AML	26	11	6	66.6% (4/6)	4 (two cycles)	1	Miscarriage (7 weeks)
2	BOT (annexectomy)	26	16 (three cycles)	9	Cycle 1 :50% (two embryos thawed) Cycle 2: 50%	1 1	1 0	Live birth (five embryos planned to be transferred)
3	BOT (annexectomy)	21	9	4	100%	4	1	Live birth
4	BOT (annexectomy)	28	13	11	100% (oneembryo thawed)	1	1	Live birth
5	AML	40	4	4	100%	4	1	Biochemical pregnancy
6	Hodgkin lymphoma	32	4	1	100%	1	0	–
7	BOT	29	6	2	100%	2	0	–
8	Breast adenocarcinoma	38	1	1	0%	0	0	–
9	Myeloma	24	2	2	100%	2	0	–
10	Thymoma	27	10	4	100%	4	0	–
11	CML	20	5	4	25% (1/4)	1	0	–

AML, acute myeloblastic leukaemia; BOT, borderline ovarian tumour; CML, chronic myeloid leukaemia.

patients for a statistically valid comparison. National data after frozen-thawed embryo transfer can be used as external reference data from a population of non-exposed subjects. However, what is important to underscore is that patients having emergency IVF have a reasonable chance of pregnancy.

We found that 52% of the French reproductive medicine centres that responded to this survey had never received a request to preserve the fertility of a female patient. We think that the true rate is probably higher, because most of the centres that did not respond to this survey were private centres or centres located at some distance from major cancer treatment centres. The response rate was higher for university teaching hospitals, 83% of which responded to our survey. Despite the legal requirement in France to inform patients about the risks of gonadotoxic treatments and possible access to fertility preservation techniques, systematic collaboration between oncologists and reproduction specialists seems to have been subject to technical difficulties. We were surprised that only 14 of the 52 IVF centres had received requests of this type from oncologists. A previous French study reported a lack of information provided to patients before gonadotoxic treatment (Mancini *et al.*, 2008). Our study is only descriptive, but the aim of this description of the French experience is to inform the community about the lack of uniformity of the practices relevant to the preservation of women's fertility in a country where such preservation is a legal obligation. Intending initially to describe the state-of-the-art concerning this technique, we discovered significantly unequal access to preserving fertility in our country. In a recent study, Armuand *et al.* (2012) showed that only 48% of women treated during reproductive age in Sweden received information about adverse effects on fertility and that only 14% received information about fertility preservation (Armuand *et al.*, 2012). Niemasik *et al.* (2012) also reported a lack of information about fertility preservation, with only 12.2% female cancer survivors informed about fertility preservation possibilities (Niemasik *et al.*, 2012).

French law authorises ART only for couples planning to have children and prohibits IVF with donor sperm for single or homosexual women. The indications for emergency IVF are still limited, and this presumably contributes to the less number of reported cycles. Proposing emergency IVF to couples (58% in this study) who did not have plans to have children before the diagnosis of the cancer is debatable. Emergency IVF may lead to ethical and legal problems, because embryo transfer will not be authorised if the couple break up or if one member of the couple dies.

Overall, 72% of the patients undergoing emergency IVF had not previously undergone chemotherapy. In ideal conditions, emergency IVF would be initiated ~20 days after diagnosis, allowing time for the patient to be informed and to consider this possibility carefully (de Ziegler *et al.*, 2010). In our study, the mean duration of stimulation was 11.2 ± 2.5 days. In situations in which anticancer treatments must be initiated urgently, as in cases of leukaemia, it is not possible to wait for a cycle of ovarian stimulation with gonadotrophins to be completed. In cases of IVF after one or two cycles of chemotherapy, the ovarian response to gonadotrophin stimulation has been shown to be poor, probably due to the destruction of growing follicles (Dolmans *et al.*, 2005). Using a short stimulation protocol, Dolmans *et al.* (2005) were unable to obtain oocytes from three patients who had previously undergone two or three courses of chemotherapy for acute leukaemia. In one patient undergoing emergency IVF after ACVBP chemotherapy for non-Hodgkin lymphoma, a single embryo was obtained and frozen from the four mature oocytes obtained. Dolmans *et al.* (2005) concluded that emergency IVF should not be offered between chemotherapy cycles to women in whom the initiation of chemotherapy could not be delayed. Ginsburg *et al.* (2001) reported that live birth rates were lower for patients undergoing IVF after anticancer treatments than for patients treated for cancer without adjuvant chemotherapy (13 versus 40%; Ginsburg *et al.*, 2001). We found that COS was possible despite a history of chemotherapy in 28% of patients: only one cycle was cancelled due to a lack of response. Unfortunately, we were unable to compare data for the cumulative dose of chemotherapy agents received before COS and the time between the last course of chemotherapy and the stimulation of ovulation. The possibility of stimulation despite a history of chemotherapy allows an additional strategy to be proposed to patients with haemopathy, including leukaemia, who have already had emergency induction treatment; this is particularly relevant in case for which ovarian cryopreservation for subsequent grafting is associated with a risk of reintroducing residual malignant disease (Dolmans *et al.*, 2010).

However, it is important to consider, and to inform patients about, the possible aneugenic and clastogenic effects of chemotherapy on the DNA of oocytes and embryos for IVF between courses of chemotherapy (i.e. carried out shortly after an initial course of chemotherapy; Meirow, 2000). In a study of mice exposed to cyclophosphamide, Meirow *et al.* (2001) found that litter size was small, with higher than normal rates of spontaneous abortion and malformation of the offspring. The frequency of malformation was highest (33%) in cases of conception with oocytes exposed to cyclophosphamide during the early stages of follicular growth (Meirow *et al.*, 2001). Several weeks after exposure had ended, congenital malformation rates fell to those found in the control group. Based on these experimental results, Meirow *et al.* (2001) recommended the avoidance of IVF for embryo freezing during or immediately after chemotherapy. Studies of cohorts of children born to mothers with a history of cancer treatment are, nevertheless, reassuring, as they suggest that the risk of chromosomal or congenital abnormalities is not higher than normal (Nagarajan and Robison, 2005; Green *et al.*, 2009; Hudson, 2010). Nevertheless, Signorello *et al.* (2006) showed that the risk of premature delivery in these patients was double that in the general population and that this risk was particularly high in cases of a history of uterine irradiation, in which 50% of children were born premature and 18.2% displayed growth retardation $<$10th percentile (Signorello *et al.*, 2006).

There have been few studies reporting results for IVF in the context of the preservation of female fertility. The only meta-analysis published on this subject included seven case–control studies, but these studies were too different for the systematic analysis recommended by the Cochrane guidelines (Friedler *et al.*, 2012). Friedler *et al.* (2012) found that fewer mature oocytes were recovered from women undergoing COS for malignant disease than from women in the control group (9.0 ± 6.5 versus 10.8 ± 6.8, $P = 0.002$). However, although only 20 couples underwent embryo transfer with thawed embryos, 50% of them obtained a live birth ($n = 10$). Thus, although smaller numbers of oocytes and embryos were obtained than in the typical context of ART, they were obtained from couples with no known history of infertility and the probability of implantation was therefore probably high. The mean number of metaphase II oocytes retrieved per patient in our study was 6.1 ± 4.2. According to Quintero *et al.* (2010), malignant disease is an independent risk factor for a poor response to COS, with an odds ratio of 5.4 (CI: 1.02–28.2; Quintero *et al.*, 2010). Rienzi *et al.* (2012) studying oocyte vitrification showed that at least eight mature oocytes have to be vitrified to

achieve a delivery rate of 46.4% (Rienzi et al., 2012). However, most studies report that the mean number of mature oocytes collected in cases of cancer is much lower than this. For this reason, we believe that embryo freezing is a valuable approach and should be included among the strategies used to preserve fertility. The experience we report may help physicians provide appropriate counselling to patients before gonadotoxic treatment.

In our study, the mean number of oocytes retrieved was 8.2 ± 4.8. The history of chemotherapy in 28% of the patients may account for the less mean number of oocytes retrieved (Ginsburg et al., 2001). Some authors have also suggested that malignant disease may have an impact on oocyte quality (Agarwal and Said, 2004), although this hypothesis remains controversial. Michaan et al. (2010) published a case–control study comparing IVF results between a group of 22 patients undergoing IVF for fertility preservation and a control group with tubal sterility (Michaan et al., 2010). They found no difference between the study and control groups in terms of the results obtained for the stimulation of ovulation and IVF. Robertson et al. (2011) also found no significant difference in the number of oocytes retrieved (12 ± 8 versus 14 ± 9) and the number of embryos cryopreserved, between 38 patients undergoing gonadotoxic treatment and couples ($n = 921$) in which the man was infertile (Robertson et al., 2011). These results conflict with those of the meta-analysis by Friedler et al. (2012) showing a statistically significant difference between the malignant disease and control groups in terms of the mean number of oocytes retrieved (11.7 ± 7.5 versus 13.5 ± 8.4). Despite the potential importance of these findings, they are difficult to compare with our results due to differences in the underlying malignant conditions between the populations studied. In this meta-analysis, the leading indication for emergency IVF was breast cancer (56.9%), with haematological indications accounting for only 14.2% of cases. The incidence of breast cancer in the meta-analysis of Friedler et al. (2012) is much higher than the incidence reported in US registry, where breast cancer represents 28% of all new cancer cases among women (Jemal et al., 2010).

When available, the parameters of the ovarian follicular reserve before COS were found to be normal, with a mean antral follicular count of 14.3 ± 8.8 ($n = 22$). In biological assessments, mean serum FSH, E_2 and AMH concentrations before COS were 6.4 ± 3 IU/l, 39.6 ± 21.1 pg/ml and 2.7 ± 2.5 ng/ml, respectively. However, the total number of oocytes retrieved was lower in our study than in the studies by Robertson et al. (2011) (12 ± 8), Pal et al. (1998) (13 ± 3) and Knopman et al. (2009) (14 ± 9), for an identical duration of stimulation. It was also lower than that reported by Sabatini et al. (2011) (11.7 ± 7.6), but similar to the values reported by Michaan et al. (2010) (8.8 ± 6.0) and Klock et al. (2010) (10 ± 6.4). Similarly, in our study, the mean number of 2PN zygotes (4.4 ± 3.3), which had a fertilisation rate of 72.3%, was lower than in the three previous studies: 6 ± 5 for Robertson et al. (2011), 5.4 ± 4.5 for Michaan et al. (2010) and 6 ± 5.7 for Sabatini et al. (2011). These differences are probably due to differences in the COS practices and protocols used by different teams.

In our study, live birth was obtained in 27% of the couples (3 babies, for 11 couples). A rate of 50% (5 babies, for 10 couples) was reported by Robertson et al. (2011) and a rate of 75% (3 babies, for 4 couples) was reported by Michaan et al. (2010). Sabatini et al. (2011) reported a lower live birth rate, of 16.7%, after one transfer, and a rate of 25% after a mean of 1.5 embryo transfers (3 babies, for 12 couples), but these values were not significantly lower than that for their group control (embryo freezing in cases of OHSS). In their meta-analysis, Friedler et al. (2012) described 20 patients who had undergone embryo transfer, with a live birth rate exceeding 50% (10 deliveries and 2 pregnancies currently underway). Thus, pregnancy rates seem to be higher in these patients than in the infertile population. This finding may be accounted for by the fertility preservation techniques being used for patients with no history of infertility. As in our study, Robertson et al. (2011) reported the occurrence of spontaneous pregnancies in some patients without the need for embryo transfer. This is probably because embryo freezing is often offered to patients undergoing chemotherapy unlikely to leave them entirely sterile, for breast cancer, for example, or after haemopathy, despite the use of high doses of cyclophosphamide (Salooja et al., 2001).

Conclusion

Long-term follow-up data for patients undergoing female fertility preservation procedures before gonadotoxic treatment remain scarce (Babb et al., 2012). Like Friedler et al. (2012), we think that multicentre studies or the creation of a national registry would provide a better assessment of the real likelihood of pregnancy after the freezing of embryos, oocytes or ovarian tissue. Data are most abundant for embryo freezing after emergency IVF (Friedler et al., 2012). A larger series of patients will need to be studied for any firm conclusions to be drawn, but our multicentre study shows that there is a real chance of pregnancy following COS and embryo freezing for malignant disease, with live birth rates similar to those for patients without malignant conditions.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

B.C. and C.P. contributed equally to this article. All co-authors participated in data acquisition from the numerous participating centres and in the revision of the article.

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None declared.

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