Feasibility of ovarian cryopreservation in borderline ovarian tumours

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Introduction

Taylor described a borderline ovarian tumour (BOT) for the first time in 1929 (Taylor, 1929). This ovarian malignancy does not exhibit overt stromal invasion at histological examination and is characterized by a less aggressive behaviour than invasive epithelial ovarian tumours (Zanetta et al., 2001). Furthermore, this tumour arises in younger patients than those who develop epithelial ovarian tumours. The role of conservative surgery aimed at preserving subsequent fertility in these young women has considerably gained momentum during the last 10 years (Gotlieb et al., 1998; Morris et al., 2000; Zanetta et al., 2001; Donnez et al., 2003; Boran et al., 2005; Fauvet et al., 2005; Morice, 2006). In these two cases (recurrence in a ‘single’ ovary in patients with a previous history of oophorectomy for BOT or bilateral involvement by serous BOT), repeating conservative surgery (using a cystectomy) with preservation of a healthy part of one ovary is not always technically feasible, particularly in patients with bulky ovarian involvement. In such cases, ovarian cryopreservation (OC) is, potentially, an excellent indication. However, there is no paper in the literature devoted to the feasibility of this procedure in patients treated for BOT. This is the aim of the present series. The end-point of the analysis was to analyse the number of cases where OC could finally be performed and to analyze the reason why this procedure was ultimately aborted.

Materials and Methods

A retrospective study was conducted to collect data concerning patients treated in our institution with the following inclusion criteria:
(1) Young patients (less than 35 years old) with a BOT are suspected because of the following: (a) previous conservative surgery of BOT or (b) morphologic appearance of the ovarian tumour on preoperative imaging. These patients could have bilateral involvement on preoperative imaging (if both ovaries are present) or unilateral involvement (in patients with a ‘single’ ovary after previous history of oophorectomy for BOT). In these patients, conservative management (cystectomy) was not considered as ‘surely feasible’ at the time of surgery (on the basis of careful analysis of preoperative imaging: abdomino-pelvic ultrasonography or magnetic resonance imaging). Two other inclusion criteria are needed:

(2) Patients seen before planned surgery in a consultation unit devoted to OC.

(3) Histologic confirmation of BOT at the time of the definitive histologic analysis. All slides had been reviewed by the same pathologist. A BOT was defined as an ovarian tumour with: (a) stratification of the epithelial lining, (b) the formation of microscopic papillary projections, (c) the presence of nuclear atypia and most of all (d) the absence of overt stromal invasion (Duvillard, 1996). The 1987 FIGO (International Federation of Gynecology and Obstetrics) classification was used for staging. Stage I is defined as a tumour limited to one or both ovaries. Stages II and III are defined as BOT with pelvic (stage II) or abdomino-pelvic (stage III) peritoneal implants. Peritoneal implants were classified as non-invasive or invasive according to the criteria previously described by Bell and Scully (1990). In the present study, the tumour stage was recorded based on the macroscopic and microscopic reports. In case of a serous tumour, the micropapillary component of the ovarian tumour was defined according to the same criteria as those used by Seidman and Kurman (1996).

Ovarian tissue cryopreservation

In cases where ovarian tissue cryopreservation had been considered feasible, the ovarian tissue was carried to the laboratory as quickly as possible. Ovary was examined and dissected by our referent pathologist who performed a macroscopic and microscopic examination of the ovary and the tumour. A frozen section analysis was carried out on the tumour to confirm the histologic subtype. The pathologist selected macroscopically thereafter a ‘healthy’ part of the ovary without disease. This part of the ovary was then sent for OC. In the absence of a healthy part of the ovary >4–5 mm (largest dimension) during the macroscopic examination, OC could not be performed. For each patient for whom OC was feasible, one sample of the ovarian cortex taken at random for cryopreservation was analysed histologically at the time of this procedure; first, to count the number of follicles and classify them and second, to verify the absence of tumour cells. The freezing protocol used was a slow cooling protocol, as previously described (Poirot et al., 2002).

Results

Patient characteristics

Data concerning 23 patients initially treated between January 2002 and February 2008 for an ovarian tumour with OC were reviewed. Six cases were excluded from the analysis. Four patients had a malignant ovarian tumour: serous in one case and mucinous in two cases, and one patient had synchronous endometrial and ovarian tumours diagnosed during the frozen section analysis. Two patients had treated conservatively benign ovarian tumours.

Finally, 17 patients had a BOT according to the frozen section analysis. In all these cases, the definitive histological analysis confirmed the results of frozen section analysis. These 17 patients constituted our study group. Median age was 24 (range, 16–34) years. Fifteen patients had a serous BOT and two a mucinous tumour.

Surgical treatment of the ovarian tumour

Fifteen patients had previously been treated conservatively for BOT: nine had undergone a unilateral salpingo-oophorectomy + contralateral cystectomy for bilateral BOT, five had undergone a unilateral salpingo-oophorectomy and one a cystectomy. Two patients had relapsed several times (one had two recurrences and one had three recurrences). These two patients had undergone OC at the time of their ‘last’ ovarian recurrence. Among the 15 patients with a recurrent tumour, the surgical procedures used on the ovary during this second surgery were salpingo-oophorectomy in 10 and cystectomy in 5 patients.

Histological analysis of the specimen removed during the management of BOT (in the first procedure in 15 patients with a previous history of BOT) demonstrated that 6 patients had stromal microinvasion and 12 patients with a serous tumour had a micropapillary pattern. Twelve patients had peritoneal implants (non-invasive in 10 and invasive in 2).

Thus, lesions were stage I in 7 patients, II in 4 (IIC in 2 patients) and III in 6 patients (IIB in 2 patients and IIIC in 1). Three patients with peritoneal implants (two of whom had invasive implants) had received adjuvant chemotherapy after the initial management of their BOT. So these three patients received their adjuvant chemotherapy before OC.

Among the 15 patients with a previous history of BOT, one (a patient with a mucinous tumour) had relapsed in the form of an invasive cystadenocarcinoma (associated with a borderline tumour) 2 years after the initial treatment of a pure mucinous BOT with stromal microinfiltration without peritoneal spread. This patient had then undergone radical surgery with pelvic and para-aortic lymphadenectomy as treatment of the recurrent disease.

Management at the time of OC

Of the 17 patients in whom OC had been planned, the procedure had been aborted in 8 of them (Fig. 1):

(1) In four patients a cystectomy was finally performed to treat the recurrent disease. As the size of the residual ovary left in situ was ‘adequate’, OC had not been performed. One of these three patients was in fact pregnant (4 weeks of gestation) at the time of treatment of the recurrence.

(2) In the patient with a mucinous BOT who had relapsed 2 years later in the form of an invasive carcinoma, OC had not been performed given the histologic subtype of the recurrence and radical surgery (hysterectomy) was performed at the time of the second surgical procedure.

(3) In three patients (18%), preservation of macroscopically normal ovarian tissue had not been feasible for cryopreservation because the whole ovary was invaded by the tumour. During
the first surgical procedure, one of these patients had undergone a salpingo-oophorectomy with a contralateral cystectomy followed by adjuvant chemotherapy.

Characteristics of the nine patients undergoing a successful cryopreservation were detailed in Table I. The median interval between the surgical resection and cryopreservation was 49 min (range, 20–85). The median number of cryopreserved ovarian fragments per patient was seven (range, 2–20). The mean size of the cryopreserved ovarian fragments was 1 cm². According to the size of the ovarian fragment sent for the histological analysis, the median ovarian cortex area was 8 (range, 5–10) mm². Only one patient had a high density of primordial and primary follicles per square millimetre (19 and 4, respectively) compared with the others with a range from 0 to 0.2 follicles/mm². One secondary follicle was found in a medulla sample in two patients. In all of them, the frozen section analysis of the ‘mirror’ part of the cryopreserved ovary had confirmed the absence of tumour in these parts of the ovary.

Discussion

Cryopreservation of ovarian tissue has developed extensively during the past 5 years and nearly 25 cases of reimplantation of ovarian fragments have been reported worldwide (Oktay and Karlikaya, 2000; Callejo et al., 2001; Radford et al., 2001; Kim et al., 2004; Tryde Schmidt et al., 2004; Meirow et al., 2005; Schmidt et al., 2005; Wolner-Hanssen et al., 2005; Donnez et al., 2006; Rosendahl et al., 2006). Five births were reported in the literature (Oktay and Tilly 2004; Donnez et al., 2004; Demeestere et al., 2006; Andersen et al., 2008). The main indications for OC are young patients (younger than 35 years) scheduled for treatment (high doses of chemotherapy, surgery or radiation therapy), which will result in impairment of ovarian function. However, such treatment should in theory be proposed to patients whose malignancy carries a relatively ‘good’ prognosis. The main current indications are haematological diseases and paediatric cancers.

In theory, one excellent indication for this management is BOT. These tumours are characterized by their onset in young patients with excellent survival (nearly 99% in stage I disease). The use of conservative management is now a gold standard for the management of early-stage BOT and this type of surgery should also be proposed to patients with non-invasive peritoneal implants (Morice, 2006). Furthermore, most of these tumours are treated exclusively by surgery, while the indications for adjuvant chemotherapy remain exceptional. Therefore, in young patients with BOT, the main issue is not to improve their survival, because these tumours carry an excellent prognosis, but rather to propose fertility-enhancing conservative management. Such tumours are characterized by a high rate of recurrence after conservative surgery, but this has no impact on survival because most of these recurrences are borderline tumours (invasive

![Figure 1](https://academic.oup.com/humrep/article-abstract/24/4/850/631739/852)
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Table 1 Characteristics of nine patients undergoing successful cryopreservation of ovarian tissue

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Size of ovarian fragments (mm)</th>
<th>Histologic results (tumour)</th>
<th>Histologic results (follicle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>32</td>
<td>Cortex: 4 x 2 x 1</td>
<td>Free of tumour</td>
<td>Cortex: 1 early primary follicle</td>
</tr>
<tr>
<td>Case 2</td>
<td>26</td>
<td>Medulla: 10</td>
<td>Free of tumour</td>
<td>No follicle</td>
</tr>
<tr>
<td>Case 3</td>
<td>24</td>
<td>Cortex: 5 x 3 x 2 mm</td>
<td>Free of tumour</td>
<td>19 primordial; 4 early primary; 1 secondary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medulla: 5 x 2</td>
<td>Free of tumour</td>
<td>Medulla: 1 secondary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortex: 15 x 2 x 1</td>
<td>Free of tumour</td>
<td>Cortex: no follicle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medulla: several of 5 mm</td>
<td>Free of tumour</td>
<td>Medulla: no follicle</td>
</tr>
<tr>
<td>Case 4</td>
<td>29</td>
<td>Cortex: 5</td>
<td>Free of tumour</td>
<td>Cortex: 1 early primary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medulla: 5</td>
<td>Free of tumour</td>
<td>Medulla: 1 secondary</td>
</tr>
<tr>
<td>Case 5</td>
<td>30</td>
<td>Cortex: 45 x 15 x 10</td>
<td>Free of tumour but endometriosis</td>
<td>Cortex: no follicle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medulla: 10 x 5 x 2</td>
<td>Free of tumour</td>
<td>Medulla: no follicle</td>
</tr>
<tr>
<td>Case 6</td>
<td>34</td>
<td>Cortex right: 30 x 30 x 10</td>
<td>Free of tumour</td>
<td>Cortex right: 25 primordial; 7 early primary; 2 primary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortex left: 15 x 3</td>
<td>Free of tumour</td>
<td>Cortex left: 40 primordial; 30 early primary; 10 primary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medulla: no follicle</td>
<td>10 primordial</td>
<td>Medulla left: 10 primordial</td>
</tr>
<tr>
<td>Case 7</td>
<td>17</td>
<td>Cortex: 6 x 5 x 3</td>
<td>Free of tumour</td>
<td>Cortex left: 3 primordial; 2 early primary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medulla: 10 x 5 x 2</td>
<td>Free of tumour</td>
<td>Medulla: 2 primordial; 5 early primary; 2 tertiary</td>
</tr>
<tr>
<td>Case 8</td>
<td>21</td>
<td>Cortex: 4 x 1 x 3</td>
<td>Free of tumour</td>
<td>Cortex: 1 primordial; 2 early primary</td>
</tr>
<tr>
<td>Case 9</td>
<td>30</td>
<td>Cortex: 4 x 1 x 3</td>
<td>Free of tumour</td>
<td>Medulla: no follicle</td>
</tr>
</tbody>
</table>

Tumours are exceptional) with an excellent prognosis which are safely cured by repeated surgery. In patients with a stage I unilateral BOT who undergo a unilateral salpingo-oophorectomy, the rate of recurrence on the remaining ovary is about 5–10% (Morice, 2006). This rate increases when patients are submitted to a cystectomy (between 30 and 45%; Morice, 2006).

When recurrences arise in a single ovary (subjects with a previous history of BOT, as in the present series), the best way to preserve fertility is to attempt repeated conservative management using a cystectomy. However, sometimes such conservative surgery is not technically feasible because the recurrence is too bulky and/or the location of the recurrence in the ovary is not accessible for repeated conservative surgery (tumour located in the centre of the ovary and not at the periphery). In order to evaluate the feasibility of repeated conservative surgery, the use of preoperative imaging (ultrasonography and magnetic resonance imaging) is a crucial step to clarify the size and the topography of the ovarian tumour. When a cystectomy is feasible (when the size of the residual ovary left in situ is sufficient for spontaneous fertility), it is the best option for fertility preservation (Fauvet et al., 2005; Morice, 2006). Several authors have reported spontaneous fertility in this context. The rationale is similar in patients with bilateral ovarian involvement during initial management (observed in 15% of serous BOT, particularly with a micropapillary pattern).

But how can fertility be improved in patients in whom preoperative imaging confirms the unfeasibility of conservative surgery? If the patient has a partner, an ‘emergency’ in vitro fertilization procedure (with an in situ tumour) can then be planned before the radical surgical procedure (removal of an ovary with uterine preservation). Such cases have been reported in the literature with successful results (Gallot et al., 2000; Fortin et al., 2007). However, the safety of such management should be evaluated. Furthermore, such management could not be proposed to patients without a partner.

In such cases, the other ‘theoretical’ option is OC. However, there are no publications on the use of OC in patients with a borderline tumour. This paper is the first devoted to this question. How can we explain this paradox? An explanation is that BOT are ovarian tumours. Consequently, several teams are reluctant to propose such management to patients with a tumour located in the organ that will subsequently be preserved because it raises the question of the safety of subsequent reimplantation of ovarian tissue fragments. This is not our point of view. BOT are probably the best indication in oncology for OC because the survival of patients is close to 100% and the prognosis of patients who develop a recurrence on a preserved ovary (in the case of conservative management) is excellent. Furthermore, the treatment of such recurrences is very simple with a single surgical resection of the recurrences and no impact on subsequent survival. These reasons explain why this management was considered and approved by our local Institutional Review Board and is now systematically proposed to young patients with a potential BOT ‘recurrence’ in a single ovary (previous history of BOT) or patients with bilateral ovarian involvement in whom preoperative radiological imaging suggests a borderline tumour.

What is then the risk of cryopreserving ovarian tissue which is ‘macroscopically’ normal but may ultimately contain microscopic borderline disease? The rate of microscopic occult ovarian tumour on macroscopically normal contralateral ovary after conservative surgery is very low. In our initial experience, we performed this procedure in 14 patients treated for BOT with macroscopically normal ovary and none of these biopsy samples turned out to be positive (Morice et al., 2001). It is noteworthy that none of the patients had
tumour during the histologic examination of the ‘mirror’ part of the cryopreserved ovary. Nevertheless, this potential risk is perhaps greater in patients with a serous BOT with a micropapillary pattern or in advanced-stage disease. Further studies are ongoing in our institution on conservative management in these two settings and may serve to evaluate this risk.

Some teams develop other procedures to avoid the risk of transmission of malignant cells from cryopreserved ovarian tissue: ovarian tissue culture with in vitro maturation (IVM) and follicle isolation. IVM has been successfully reported in a patient of 43 years with a borderline tumour (Huang et al., 2007), but the number of oocytes obtained after such procedures is low.

The results of the present series demonstrated that, even if OC is planned, it is not always technically feasible. The first reason for abandoning this procedure was the fact that a cystectomy was finally carried out during the surgical procedure and the OC was then abandoned. Such decision depended on the conviction of the physicians. But as mentioned above, conservative surgery is the best fertility preservation option which should be used first. In present series, in four patients with absence of contralateral ovary (previous history of BOT) cystectomy was finally technically performed. In these cases, we have decided not to remove macroscopically normal remaining ovarian tissue (that was small after cystectomy) for ‘potential’ OC in order not to reduce the size of the remaining ovary and therefore not potentially impair subsequent fertility.

The second reason for abandoning the OC is the discovery of a malignant recurrence in a patient with a previous history of a mucinous BOT. Such cases are exceptional. Less than 10 similar cases have been reported in the literature (stage I disease) among more than 1200 cases of conservative management published (Salomon et al., 2006). Such rare events are perhaps more commonly associated with mucinous BOT. In these exceptional cases, it is not possible to cryopreserve a malignant ovary.

Finally, the last reason for aborting OC is a technical reason: even if the pathologist tries to select macroscopically normal ovarian tissue fragments for OC, this is sometimes impossible because the whole ovary is invaded by the tumour. This case was observed in three of our patients and is a true failure in this context but there is no way of avoiding such a situation.

The next step would be to define the minimum size of the ovarian tissue sample that should be preserved to yield a given number of follicles after thawing. However, the limits of the ovarian sampling are tumour invasion and enough remaining healthy tissue to enhance spontaneous fertility. In our study, the number of cryopreserved fragments was therefore varied. Follicle density was high in only two cases. This low follicle density could be explained by the fact that, as the size of the ovarian specimen sent for OC is sometimes small, the ovarian specimen selected for histological analysis was not the most macroscopically ‘healthy’ ovarian fragment because such fragments are directly sent for cryopreservation. So this low follicle density determined during histologic analysis is probably not an exact reflection of the follicle density in the cryopreserved fragments. In addition, as we decided to cryopreserve the maximum amount of ovarian tissue for potential future use, we did not analyse the number of surviving follicles after cryopreservation and thawing. At the present time, no one knows how many fragments will be necessary to restore fertility. This is why the fragments we submit for histologic analysis are small and, probably, not strictly representative of the ovarian tissue since our objective is to preserve the best part of ovarian tissue. Nevertheless, a higher follicular density is correlated with a greater chance of restoring fertility.

In conclusion, these results suggest that OC should be considered in young patients with a BOT in whom conservative surgery is not technically feasible (recurrent tumour in a single ovary or ‘massive’ initial bilateral ovarian involvement). In the present series, in 18% (3/17) of the cases in which this procedure was planned, it was unfeasible because of massive ovarian involvement and it was impossible to macroscopically select ‘normal’ ovarian tissue.

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**References**


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