Objective. To describe a case series of seven women with SLE and other systemic autoimmune rheumatic diseases (SARDs) who required cyclophosphamide therapy and underwent fertility preservation treatments.

Methods. Of the seven patients reported here, five women had SLE with nephritis, the sixth had immune thrombocytopenia purpura (ITP) and the seventh had microscopic polyangiitis (MPA) with renal involvement. All women were nulliparous and younger than 35 yrs.

Results. Patients with SLE underwent in vitro maturation (IVM) of immature oocytes aspirated during a natural menstrual cycle followed by vitrification of the matured oocytes if a male partner was not available, or vitrification of embryos if one was available. The patient with ITP and the patient with MPA underwent gonadotropin ovarian stimulation followed by oocyte or embryo vitrification. All women completed fertility preservation treatment successfully and mature oocytes or embryos (36 and 13, respectively) were vitrified. No complications were associated with this treatment and cytotoxic therapy was initiated as scheduled in all cases.

Conclusions. Oocyte or embryo cryopreservation should be considered for fertility preservation in young women with SARDs who face imminent gonadotoxic treatment. In patients, where gonadotropin ovarian stimulation is deemed unsafe, IVM of immature oocytes, aspirated during a natural menstrual cycle, followed by vitrification or fertilization of the mature oocytes, seems to be safe and feasible. For patients in whom hormonal ovarian stimulation is not contraindicated, this method may be considered depending on the urgency to start cytotoxic therapy.

KEY WORDS: Systemic lupus erythematosus, Fertility preservation, Vitrification, In vitro maturation, Systemic autoimmune rheumatic diseases.

Introduction

SLE as well as other systemic autoimmune rheumatic diseases (SARDs) are common in women of childbearing age. The prognosis for patients with SLE has greatly improved over the past few decades with between 80% and 90% of all patients surviving at least 10 yrs [1]. Cyclophosphamide remains the drug of choice for selected SLE manifestations such as severe renal [2] or extra-renal [3] involvement. In addition, cyclophosphamide is still frequently used for a number of other SARDs such as vasculitis [4], SSc [5, 6] and multiple sclerosis [7]. However, the impact of cyclophosphamide treatment on future fertility is an important consideration for many young women as it can cause premature ovarian failure (POF).

Fertility preservation treatments for cancer patients have been widely reported [8]. Surprisingly, this has not been the case for young women with autoimmune disease. Gonadotropin releasing hormone (GnRH) agonist injections administered during the course of cytotoxic therapy have been suggested to reduce ovarian damage to some extent [9], but several studies have cast doubt on the efficacy of this approach [10]. Other available fertility preservation treatments, such as embryo or oocyte cryopreservation, have not been reported yet for patients with SARDs. This might be due in part to the concerns about the safety of elevated serum oestrogen levels that are associated with various hormonal ovarian stimulation protocols necessary for in vitro fertilization (IVF).

Herein, we are the first to report on seven young women with SARDs exacerbation who vitrified their oocytes or embryos for the purpose of fertility preservation prior to cyclophosphamide treatment.

Materials and methods

This study involves an analysis of seven young women requiring cyclophosphamide therapy for severe autoimmune disease manifestation. All had been referred to the McGill Reproductive Center (MRC), Montreal, Canada, a university-based, tertiary medical centre, by their primary physician (rheumatologist or hematologist) to consider fertility preservation prior to initiation of cytotoxic therapy. Each patient was evaluated and consulted with a reproductive endocrinology and infertility (REI) specialist and a qualified coordinating nurse regarding the various fertility preservation options available. A thorough evaluation included a detailed history, physical and gynaecological examination, pelvic ultrasound scan, complete blood count (CBC), serum electrolytes, glucose, urea, creatinine, liver enzymes and prothrombin time (PT) and partial thromboplastin time (PTT). In addition, the patients were examined and consulted with an anaesthesiologist regarding the various anaesthesia options available during oocyte aspiration. The hospital ethics committee approved fertility preservation treatment and all women signed an informed consent prior to treatment. The chart review was approved by the hospital’s Director of Professional Services.

Our strategies for fertility preservation treatment are the following: if there are time constraints or contraindications to hormonal ovarian stimulation, we perform immature oocyte retrieval in a natural menstrual cycle, mature the oocytes in vitro and vitrify either embryos, when a male partner is available or oocytes if one is not. In cases with no time constraints and no contraindications to gonadotropin ovarian stimulation, we perform hormonal ovarian stimulation followed by oocyte retrieval and vitrification of embryos or mature oocytes, according to the availability of a male partner. Owing to the concerns relating to the elevated serum oestrogen levels associated with hormonal ovarian
stimulation, the five SLE patients underwent immature oocyte collection during a natural cycle followed by in vitro maturation (IVM) of oocytes.

In general, the IVM procedure is performed at the MRC as follows. The antral follicle count (AFC) (dimension of 2–10 mm) in both ovaries is determined by an ultrasound scan that is performed at the first consultation [11]. In amenorrhoeic women, withdrawal bleeding is induced with a course of progesterogens and oocyte aspiration is scheduled for early- to mid-follicular phase of the following cycle. Patients are given one subcutaneous injection of 10 000 IU of human chorionic gonadotropin (hCG) 36–38 h prior to collection [12, 13]. Oocytes are retrieved with a specially designed 19-gauge single-lumen aspiration needle (K-OPS-7035-RWH-ET, Cook, Australia) under transvaginal ultrasound guidance. Oocyte maturity is determined microscopically by the presence of a germinal vesicle in the cytoplasm or extrusion of the first polar body into the perivitelline space. Mature oocytes are either inseminated by intracytoplasmic sperm injection (ICSI), if a male partner is available, or vitrified immediately if one is not. Immature oocytes are matured in an Organ Tissue Culture Dish (60 x 15 mm; Falcon, NJ, USA) containing 1.0 ml of IVM-Medium (Coopersurgical/Sage, CT, USA) supplemented with a final concentration of 75 mIU/ml of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) at 37°C in an atmosphere of 5% CO2 in air with high humidity. Oocyte maturation is assessed after 24 h in IVM culture medium, and matured oocytes are either inseminated by ICSI or vitrified. Any remaining immature oocytes are further cultured for another 24 h (total of 48 h), and any additional mature oocytes are similarly inseminated or vitrified.

The patient with ITP required cytotoxic therapy for severe manifestation of her disease. Both she and the patient with microscopic polyangiitis (MPA) underwent hormonal ovarian stimulation with the approval of their primary physicians. The mature oocytes that were aspirated were vitrified or inseminated with their partners’ sperm and the embryos then vitrified.

Vitrification of mature oocytes and embryos

Mature oocytes or embryos were suspended in equilibration medium containing 7.5% (v/v) ethylene glycol (EG) plus 7.5% (v/v) 1,2-propanediol (PROH) for 5 min at room temperature, and then transferred to vitrification medium containing 15% (v/v) EG plus 15% (v/v) PROH plus 0.5 M sucrose at room temperature for 45–60 s. They were loaded on a specially designed vitrification device, the McGill Cryoleaf (Medicult, Jyllinge, Denmark), and plunged immediately into liquid nitrogen for storage.

### Results

From 2005 to 2007, seven patients with SARDs underwent fertility preservation treatment at the MRC. All patients were younger than 35 yrs and required cyclophosphamide therapy for a severe manifestation of their disease. Five women had SLE with renal involvement; one had MPA with focal segmental necrotizing glomerulonephritis (GN) and one had severe ITP. Five women were single; therefore, oocyte vitrification was offered. For the two patients who had a male partner, embryos were vitrified. Owing to the concerns relating to the elevated serum oestrogen levels associated with hormonal ovarian stimulation, SLE patients underwent immature oocyte collection during a natural cycle followed by IVM of oocytes. The immature oocytes were matured in vitro. The women with SARDs other than SLE underwent ovarian stimulation protocol using recombinant FSH with GnRH antagonist suppression [14]. For the women who underwent IVM treatment, the median interval from the first visit at the MRC to the day of oocyte collection was 7 days (range 2–11 days) (Table 1). This extremely short period is tremendously important in patients requiring rapid initiation of cytotoxic therapy and is made possible by the flexibility of IVM treatment compared with conventional hormonal stimulation protocols. Fertility preservation treatment characteristics are summarized in Table 2. All women completed fertility preservation treatment successfully and mature oocytes or embryos (36 and 13, respectively) were

### Table 1. Characteristics of seven women with autoimmune diseases who underwent fertility preservation treatment prior to cytotoxic therapy

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Disease</th>
<th>Marital status</th>
<th>Planned cytotoxic treatment</th>
<th>Menstrual cycle length (days)</th>
<th>Interval from first visit to oocyte aspiration (days)</th>
<th>Fertility preservation treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>SLE nephritis</td>
<td>Single</td>
<td>Cyclophosphamide</td>
<td>28</td>
<td>7</td>
<td>IVM</td>
</tr>
<tr>
<td>25</td>
<td>SLE nephritis</td>
<td>Single</td>
<td>Cyclophosphamide</td>
<td>30</td>
<td>9</td>
<td>IVM</td>
</tr>
<tr>
<td>26</td>
<td>SLE nephritis</td>
<td>Single</td>
<td>Cyclophosphamide</td>
<td>28</td>
<td>11</td>
<td>IVM</td>
</tr>
<tr>
<td>19</td>
<td>SLE nephritis</td>
<td>Single</td>
<td>Cyclophosphamide</td>
<td>28</td>
<td>2</td>
<td>IVM</td>
</tr>
<tr>
<td>35</td>
<td>SLE nephritis</td>
<td>Married</td>
<td>Cyclophosphamide</td>
<td>30</td>
<td>7</td>
<td>IVM</td>
</tr>
<tr>
<td>26</td>
<td>MPA and focal segmental necrotizing GN</td>
<td>Single</td>
<td>Cyclophosphamide</td>
<td>28</td>
<td>11</td>
<td>Gonadotropin stimulation</td>
</tr>
<tr>
<td>30</td>
<td>ITP</td>
<td>Married</td>
<td>Cyclophosphamide</td>
<td>28</td>
<td>28</td>
<td>Gonadotropin stimulation</td>
</tr>
</tbody>
</table>

### Table 2. Fertility preservation treatment characteristics of seven women with autoimmune diseases prior to cytotoxic therapy

<table>
<thead>
<tr>
<th>Age</th>
<th>Disease</th>
<th>Fertility preservation treatment</th>
<th>Antral follicle count (AFC)</th>
<th>Total no. of oocytes aspirated</th>
<th>No. of MII stage oocyte at day of collection (in vivo)</th>
<th>No. of mature oocytes matured in vitro</th>
<th>No. of mature oocytes/embryos vitrified</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>SLE nephritis</td>
<td>IVM</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>SLE nephritis</td>
<td>IVM</td>
<td>23</td>
<td>10</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>26</td>
<td>SLE nephritis</td>
<td>IVM</td>
<td>34</td>
<td>19</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>19</td>
<td>SLE nephritis</td>
<td>IVM</td>
<td>14</td>
<td>18</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>35</td>
<td>SLE nephritis</td>
<td>IVM</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2 embryos</td>
</tr>
<tr>
<td>26</td>
<td>MPA and focal segmental necrotizing GN</td>
<td>IVM</td>
<td>9</td>
<td>13</td>
<td>6</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>ITP</td>
<td>GnRH antagonist protocol with FSH 150 IU/day</td>
<td>9</td>
<td>20</td>
<td>11</td>
<td>4</td>
<td>11 embryos</td>
</tr>
</tbody>
</table>
vitrified (Table 2). No complications occurred following the oocyte aspiration procedure and all patients were able to initiate their cytotoxic therapy regimen, as scheduled.

Discussion

SLE and other autoimmune diseases affect women of reproductive age. These diseases occasionally require the use of gonadotoxic agents such as cyclophosphamide for treatment of severe manifestations. Therefore, a significant number of young women with SARDs may be exposed to gonadotoxic therapies, which may lead to POF and infertility.

Cyclophosphamide is an alkylating agent that acts by transferring alkyl groups to biologically important cellular constituents. The mechanism involved in the loss of primordial follicles in response to cyclophosphamide administration is not well understood. Several animal as well as human studies demonstrate that cytotoxic agents may damage ovarian pregranulosa cells [15] and cortical blood vessels [16] and that oocyte and follicle loss is associated with apoptosis [17]. A regimen of intermittent monthly boluses of intravenous cyclophosphamide infusions of 0.75–1 g/m² remains an accepted treatment for severe diffuse proliferative lupus nephritis [2, 18, 19]. The frequency of ovarian insufficiency secondary to cyclophosphamide administration has been shown to be increased in young patients and correlates with the cumulative dose [20]. Half of the treated women, aged 32 yrs or older, who undergo treatment, will develop ovarian failure after a cumulative dose of 8 g/m², and 90% will have ovarian failure following 12 g/m² [21].

Fertility preservation treatments are widely reported for various cancer patients facing chemotherapeutic and radiotherapy. Fertility preservation options that are currently available include embryo, ovarian tissue [22] or oocyte cryopreservation [8]. Embryo cryopreservation is the only established female fertility preservation strategy. However, it is not feasible for young women who have no male partner. Another alternative is ovarian tissue cryopreservation, which offers the special benefit of providing oestrogen activity following ovarian autotransplantation. However, this option requires two surgical procedures: one to excise the ovarian tissue and the second for autografting. Moreover, the survival time of the ovarian graft following transplantation may be limited [23]. Oocyte cryopreservation was first reported by Sherman and Lin [24] using unfertilized mouse oocytes. Nearly two decades later, the first live offspring from frozen–thawed mouse oocytes were reported by Parkening et al. [25] and Whittingham [26], both using the slow freezing/rapid thawing method. Subsequently, this method was implemented for human oocytes; however, most resulting pregnancies were published as case reports or series of small numbers of patients. There have been only two large studies reported on human oocyte cryopreservation using this method [27, 28]. In a recent meta-analysis, the clinical pregnancy rate following transfer of embryos derived from slow frozen/rapid thawed oocytes was estimated to be ~25% per cycle [29].

A relatively new alternative for oocyte cryopreservation is the vitrification procedure. Vitrification is the ice-free solidification of an aqueous solution by ultra-rapid cooling. This technique appears to be more effective than the conventional slow freezing procedure [30]. Several authors have reported marked improvement in oocyte survival, implantation and clinical pregnancy rates [31, 32]. At the MRC, use of the vitrified–thawed oocyte treatment has yielded a survival rate of over 80%, a fertilization rate of 75% and a clinical pregnancy rate of 45% per cycle [33]. Vitrification is indeed a promising method for oocyte cryopreservation, and it offers a good opportunity for young women requiring fertility preservation for cancer as well as other conditions [34].

Mature oocytes for vitrification can be aspirated from the ovaries following gonadotropin ovarian stimulation using FSH and LH preparations. However, in cases of oestrogen-sensitive tumours or autoimmune disease, especially SLE, the high levels of oestrogen associated with hormonal ovarian stimulation may not be safe. The association between oestrogen and SLE is a matter of conflicting evidence. Some reports [35], but not others [36], suggest that elevated oestrogen levels may exacerbate SLE. An early report [37] raised the possibility that elevated serum oestrogen levels during hormonal ovarian stimulation might induce SLE flares. Larger studies, however, did not support these concerns [38, 39]. Owing to this conflicting evidence, most physicians are reluctant to use hormonal ovarian stimulation for SLE patients, especially during disease exacerbation. Moreover, SLE patients with aPLs are already at high risk of thromboembolic events, a risk that would surely increase with hormonal ovarian stimulation. In other SARDs, it is becoming clear that there also exists an increased risk of thrombotic events [40, 41] that are usually highest at the time of active disease. It could thus be argued that for most patients with SARDs requiring cyclophosphamide therapy, the use of hormonal ovarian stimulation may be contraindicated. There have been reports regarding modified hormonal stimulation protocols, using aromatase inhibitors along with FSH preparations, in order to limit the increase in serum oestrogen levels during ovarian stimulation treatments in breast cancer patients [42]. However, the peak serum oestradiol levels using this protocol are still much higher (mean 1744.6 ± 1023.8 pmol/l) [43] compared with the ones measured during IVM cycles (270.6 ± 90.5 pmol/l).

In women with a contraindication to hormonal stimulation or with time constraints, immature oocytes can be aspirated during the natural menstrual cycle without any hormonal stimulation. These immature oocytes can be matured in vitro following incubation in a special culture media and subsequently vitrified [8]. We have recently showed that oocytes that were matured in vitro and vitrified resulted in a birth of a healthy offspring [44]. In extreme cases, when the gonadotoxic treatment is imminent, oocyte aspiration can be performed on any day of the menstrual cycle, including the luteal phase, to avoid any delay in the treatment [45]. At the MRC we have recently reported a live birth rate of 20% using vitrified–thawed immature oocytes [33]; therefore, this method of fertility preservation seems safe and reasonably successful.

Cancer as well as SARDs patients may have impaired physical and physiological conditions prior to cytotoxic or radiotherapy. This emphasizes the necessity of a thorough and comprehensive evaluation by a multidisciplinary team prior to fertility preservation treatment. Moreover, fertility preservation service must be flexible and available at a short notice due to the possibly short interval from the decision to the initiation of cytotoxic therapy. (Having said that, it should be emphasized that early referral, for fertility preservation consultation, of cases where cyclophosphamide is an option in treatment in the future, can significantly improve success rate.)

Pregnancy carries a risk of SLE flare-up and other pregnancy-associated complications in women with SLE. Therefore, following remission, a multidisciplinary team evaluation and follow-up is essential prior to a planned pregnancy. A favourable pregnancy outcome is expected with proper perinatal care [46, 47]. In extreme cases, when pregnancy is contraindicated, the cryopreserved oocytes or embryos can be combined with surrogacy.

In conclusion, oocyte or embryo cryopreservation should be considered for fertility preservation in young women with severe SARDs manifestations requiring imminent gonadotoxic treatment. In patients where gonadotropin ovarian stimulation may not be safe or in a situation where cytotoxic therapy must be started urgently, IVM of immature oocytes aspirated during a natural menstrual cycle, followed by fertilization or vitrification of the mature oocytes, seems to be safe and feasible. For other situations, gonadotropin ovarian stimulation followed by oocyte aspiration and vitrification may be considered. Fertility
preservation can help these young women cope better emotionally with their cytotoxic therapy, as it provides them with the hope of being able to have a biological child in the future.

Rheumatology key message

- Oocyte or embryo cryopreservation should be considered for fertility preservation in young women with severe SARDs manifestations requiring imminent gonadotoxic treatment.

Disclosure statement: The authors have declared no conflicts of interest.

References


