Chapter 1

Why freeze human eggs? Clinical and biological indications for human oocyte cryopreservation

Ethical and legal indications

In recent years, knowledge of mammal embryo cryobiology has increased considerably. In the human field, embryo cryopreservation has been prompted by the surplus embryos (embryos not immediately used for reproduction) generated during in vitro fertilization programs and embryo transfer (IVF-ET). Since the beginning of extracorporeal fertilization, human embryo freezing has become a requirement demanded by the human fertility therapy procedure. Initially, because of low embryo implant rates, procedures that stimulated many follicles were used to obtain many oocytes and consequently generate many embryos to make up for their lack of vitality. This strategy increased not only pregnancy rates, but also the number of multiple pregnancies and associated complications. Scientific and technological developments led to an improvement in embryonic viability and it was decided to reduce the number of transferred embryos to a maximum of two or three, which is currently the number generally accepted by the scientific community. The generation of an excessive number of embryos gradually appeared unsuitable. The only solution seemed to be that of freezing those in excess, a technique which was successfully attained. However, embryo storage meets with intrinsic ethical reservation on the part of some couples who prefer the number of oocytes for insemination to be reduced to a minimum. Generally speaking, ethical concerns are also present among researchers and the wider public, so much so that, in some countries such as Germany and Italy, this technique has been strongly restricted and sometimes even forbidden.

Apart from ethical reservation, embryo cryopreservation can cause legal issues. Frozen embryos belong equally to the two people who generated them and who may decide, in time, to divorce. In this case, the fate of the embryos is uncertain and will depend on the decision of a court of law. Oocyte freezing would avoid all these ethical and legal problems.

Since the announcement of the first pregnancy from cryopreserved embryos (Trounson and Mohr, 1983), embryo cryopreservation has been widely used and it has accounted for a significant percentage of the treatments of medically assisted procreation. In 2002 the "National Summary of Fertility Centers Report" (NSFCR) reported that 97% of the 391 US infertility clinics offered the opportunity to cryopreserve embryos. About 17% of treatments of medically assisted procreation involve the transfer of frozen embryos. In 2002 more than 400 000 embryos were cryopreserved in the United States. A similar increase in the use of embryo cryopreservation also occurred in Europe and Australia. It was estimated that there were 52 000 frozen embryos in the United Kingdom in 1996, 71 000 frozen embryos were reported in Australia and New Zealand in 2003 (Hoffman et al., 2003) and 15 000 frozen embryos were reported in Canada in 2003 (Newton et al., 2007).
Studies suggest that the storage of surplus embryos is progressively increasing because of the tendency to reduce the number of transferred embryos and the improved success rates of medically assisted procreation techniques. The rapid increase in the use of the cryopreservation method has not allowed us to acquire an adequate awareness of the psychological, social and legal consequences of its use. Moreover, an accurate assessment of the "destiny" of the frozen embryos is necessary because of their progressive increase. There are several choices: the embryos can be used in new in vitro fertilization (IVF) cycles, they can be donated to another infertile couple, they can be used in research or they can be destroyed. The couple can also choose to extend the length of time the embryos remain frozen beyond the deadline, which varies depending on the country.

The great majority of the embryos (88%) are cryopreserved in order to be used in a subsequent cycle. Only 2% of the embryos are cryopreserved in order to be donated to other couples, or they are destroyed (Bankowski et al., 2005). In Klock's (2001) study 107 couples out of 404 (26%) had remaining frozen embryos three years after the treatment and only 17 of those couples were still in treatment. Fifty-two out of 91 couples (57%) responded to the center's analyses: 17 of these 52 (33%) chose to destroy the embryos, 7 (13%) chose to donate to an infertile couple, 5 (10%) to donate to research, 15 (29%) to extend the cryopreservation and 2 (4%) were undecided (Bankowski et al., 2005). Some centers have asked couples to choose the embryo's destiny before IVF treatment to avoid possible unnecessary storage of frozen embryos. These preliminary choices do not take into account the factors which can occur during and after treatment. The validity of a preliminary consensus is questionable because the request is not associated with the inability of the couple to express a consensus at a later time but rather with the organizational needs of the center. The possibility of choice should be provided to the couple. The preliminary consensus should be reserved only for the cases in which one or both spouses are unable to make a decision. The only exception to this rule are cases in which a certain provision concerning the embryos is an essential condition of access to the treatment for one of the two partners (Penning, 2002; Newton et al., 2007).

Many couples change their minds about the embryo's destiny during treatment. This change is associated with the outcome of the treatment and it confirms the need for a dual informed consensus. In fact in Klock's (2001) study only 12 of 41 couples (29%) confirmed the choice expressed before treatment. Newton et al. (2007) evaluated the changes in the choice of the couples and correlated them with the treatment outcomes. Thirty percent of the examined couples had not used the embryos after five years and 31% of these couples had not updated their provisions about the embryo's destiny. The couples who had achieved a pregnancy were more likely to provide new indications and to choose to destroy the embryos rather than donate them for research. Fifty-nine percent of the couples changed their mind about the destiny of their embryos after the treatment (Table 1.1) (Newton et al., 2007).

These data show that the majority of embryos are destined to never be transferred into the uterus in the absence of a specific legislation for the protection of the embryos. In the United States the majority of people consider an embryo as an inanimate biological entity that cannot enjoy human rights. Therefore a change in US law to modify the legal status of the embryo does not seem realistic. The only federal restriction regards the government support in the creation and destruction of the embryos. The amendment "Dickey-Wicker" forbids the use of federal funds in case of creation, destruction or damage to embryos. The laws change depending on the State: four States regulate the donation of frozen embryos and two States govern their adoption. Among these six, only Louisiana provides protection.
for the embryo, while the other States only provide protection for the couples willing to donate or adopt embryos. In fact, only in Louisiana is an embryo considered as a “human being” with the dignity and the rights of a legal person. Therefore, the creation of embryos is only allowed for transferring them to the uterus. However, the majority of States do not have a specific definition of “embryo” and they accept the definition of the High Court of the United States: “the unborn has never been recognized in the law as a person in the whole sense.”

The Courts have often considered the embryos like property. In a Tennessee case (1992) a divorced couple asked the court to legislate on the destiny of their frozen embryos because the father wanted to destroy them while the mother wanted to transfer them to her uterus or to donate them to another couple. The trial judge considered the embryo as a human being and supported the transfer of the embryos into the uterus, but the Court of Tennessee changed this decision; they did not consider the embryo as a human being and supported the destruction of the frozen embryos. Similarly in the case Kass vs. Kass (1998) the status of human being of the embryo was denied and the law supported the spouse who wanted to avoid the pregnancy. A similar sentence was issued in 2000 to reject a previously signed agreement giving the custody of the embryos to the wife in case of divorce. In 2003 the Supreme Court of Iowa affirmed that the State only has to take care of the welfare of already born children and not of any fertilized oocytes not yet implanted.

In Europe, various provisions relating to embryo cryopreservation exist. In Germany the “Embryo Protection Act” (1990) states that more than three embryos per cycle of IVF cannot be created and that all embryos must be transferred to the uterus; embryo freezing and destruction are forbidden. The “French Bioethic Law” (1994) forbids embryo production for carrying out research and for obtaining stem cells. A maximum number of embryos which can be created is not established and embryo freezing is not forbidden. Also in Switzerland, there is a law that prohibits the creation of embryos for research purposes in order to protect the legal rights of the human embryos; however, research is allowed on any supernumerary embryos (Brugger, 2009).
Before the present legal regulation, in Italy embryo cryopreservation was carried out in a few centers; in 2003 there were 74 centers (37.4%) offering embryo cryopreservation.

The introduction of the Law 40/2004 reduced significantly the number of embryos stored in the centers. In fact the Law establishes that each IVF treatment must produce no more than three embryos to be transferred into the uterus. In 2000, the number of embryos stored in the assisted reproductive technology (ART) centers was 24 452. In 2007 the Italian National Institute of Health finished the first phase of the count of the abandoned embryos in the centers: only 8 out of 88 surveyed centers affirmed having no frozen embryos stored. Fifty-four (67.5%) centers declared having abandoned frozen embryos while the remaining 26 centers (32.5%) reported having no abandoned frozen embryos. Therefore, in 2007, the total number of abandoned frozen embryos was 3415 belonging to 825 couples.

Since 2003, the number of embryo thawing cycles gradually decreased from 3102 to 704 in 2007, that is 1.6% of the ART cycles in Italy. Instead, the number of oocyte freezing and thawing cycles progressively increased from 102 in 2003 to 2994 in 2007. However, oocyte cryopreservation is still performed in relatively few centers. In 2006 and 2007 the centers that have not performed any oocyte freezing cycles comprised 43.5% and 40.9% of centers, respectively. In 2006 in the Italian centers, 32 860 oocyte pickups were performed and 223 359 oocytes were recovered. The average number of recovered oocytes per cycle was 6.8. Of these only 38.8% were inseminated, 12.9% was cryopreserved, while 107 832 oocytes (48.3%) were rejected. In 2007 the situation was similar: 35 645 oocyte pickups were performed and 234 004 oocytes were recovered (the average was 6.6 oocytes per cycle). Of these 38.3% (89 645 oocytes) were inseminated, with an average of 2.5 oocytes per sample, 11.8% (27 513 oocytes) were cryopreserved (0.8 per cycle) and 49.9% (116 846 oocytes) was rejected (3.3 oocytes per cycle).

In 2009, the High Court of Italy (Act 151/2009) eliminated the prohibition of generating more than three embryos. The new limitation established that the number of produced embryos should be that strictly necessary to be transferred. On the other hand, the High Court did not cancel the prohibition of embryo freezing, except in cases of patient health risks. In actual fact, the interpretation of this rule by a number of ART centers led to a new, huge increase in the number of frozen embryos with potentially uncertain destiny. Therefore, storing eggs instead of embryos should be taken into consideration.

Fertility-saving indications

In the last 30 years, new cancer treatments have improved patient survival rates. The survival rate for all types of tumors is 50%; 90% for Hodgkin’s disease, 4–67% for acute lymphocytic leukemia and 33–77% for Wilms’ disease diagnosed in childhood. Survival of breast cancer patients has reached 70–75% (National Cancer Institute, 1973–1987). Beyond survival, the quality of a patient’s life has now become a key issue. Alterations of reproductive functions in cancer patients depend on both the underlying tumor type and ensuing therapy. It is well known that antiblastic drugs and radiotherapy cause serious damage to the gonads, in many cases inducing premature ovarian failure. Moreover, several studies have shown that the neoplasm itself can reduce fertility (Nieto et al., 1999; Bahadur, 2000). Pal et al. (1988) reported that patients with neoplastic pathologies had lower ovarian quality compared with controls. Although the number of oocytes collected was the same for both groups, oocyte quality, mature oocyte and fertility rates were lower among oncology patients.
Structural and ultrastructural research has shown gonad alterations among patients receiving chemotherapy to be fairly heterogeneous. Derangement depends on patient age; when therapy was started; and the type, dosage and duration of cancer therapy (Nicosia et al., 1984; Marcello et al., 1990). Observational evidence indicates that, after chemotherapy, the ovaries of prepubertal adolescents are less affected than those of older patients (Meirow, 2000). In a retrospective study carried out in cooperation with the Rizzoli Orthopaedic Institute of Bologna, similar findings were reported. This study followed the reproductive function of 92 women who had received chemotherapy. Sixty-nine percent of the 92 women had amenorrhea during chemotherapy and 2 of these (all post pubertal at the time of cancer therapy) presented premature ovarian failure (permanent amenorrhea) (Longhi et al., 2000).

Both pharmacological and surgical therapy show significantly increased survival of cancer patients (McVie, 1999); consequently, an increasing number of patients face a life of impaired fertility induced by cancer treatment. Several preventive tools have been proposed to preserve reproductive capacity. Ovarian tissue cryopreservation has been investigated in the past few years as a fertility-saving procedure (Oktay et al., 2004), and the first live birth has been reported after auto-transplantation of ovarian grafts (Donnez et al., 2004). The risk of reintroducing neoplastic cells with this procedure must, however, be kept in mind. On the other hand, in vitro maturation of early immature follicles in tissue presently appears to be not yet reliable (Gook et al., 2004). Oocyte harvesting, fertilization and subsequent embryo storage have also been proposed for partnered women. However, ethical and pragmatic considerations should be taken into account in this choice.

Oocyte storage may be an alternative which does not raise ethical concerns. It might be considered the ideal strategy for preserving the fertility of cancer patients provided they can postpone chemotherapy. This option is feasible in several neoplastic conditions as well as for patients with severe endometriosis and genetic premature ovarian failure. The American Society of Clinical Oncology and the American Society for Reproductive Medicine (ASRM) have issued guidelines recommending that the potential effects of cancer treatment on fertility and the options to preserve fertility should be presented to the patient in the initial stage of treatment (The Ethics Committee of the American Society for Reproductive Medicine, 2005; Lee, 2006).

With heightened public awareness, more aggressive screening and treatment innovation, survival rates for cancer patients are anticipated to increase steadily, allowing more patients to look forward to a normal life after cancer. Unfortunately, radical surgery and chemo- and/or radiotherapy can leave patients infertile or sterile. Goodwin et al. (1999) estimated that 53–89% of breast cancer patients treated with chemotherapy undergo premature menopause; the risk for premature menopause was found to be strongly associated with the age of the patient and the use of systemic chemotherapy. In a study of young cancer patients, Meirow (2000) found that the use of alkylating agents, such as cyclophosphamide and busulfan, frequently used in childhood cancer, had an odds ratio (OR) of 4.0 for ovarian failure, which is significantly higher than platinum agents (OR: 1.8), plant alkoids (OR: 1.2) or anti metabolites (OR: <1). The radiation damage is dose-related and depends on fractionation schedule and irradiation field. In women the severity of damage is also influenced by age at the time of exposure to radiotherapy; younger women have, in fact, a greater reserve of primordial follicles than older women and they may thus have a higher remaining primordial pool after a cancer treatment (Wallace et al., 2005).
Oocyte cryopreservation has become a potentially good option for preserving female fertility, particularly for oncological patients, as was documented for the first time in 2004. Porcu et al. (2004) reported data regarding cryopreservation of oocytes from young women who had to undergo cancer treatments. In these young oncological patients, the ovarian stimulation was found to be very efficient, with the retrieval and storage of an average of 15 oocytes per patient that can lead to about two embryo transfers after recovery from the malignancy (Table 1.2).

The first births recently reported demonstrate that oocyte cryopreservation in oncology is a reliable option. Frozen oocytes of a cancer patient were first used in a gestational carrier (Yang et al., 2007), who conceived with frozen oocytes belonging to a patient with Hodgkin’s lymphoma that were retrieved and cryopreserved before radiotherapy.
commenced. Ten out of twelve cryopreserved oocytes survived after thawing and nine were fertilized and developed into good quality embryos, which were transferred in three different cycles to a gestational carrier. A singleton pregnancy was achieved after the third transfer, resulting in the delivery of a healthy male. However, this result was reached thanks to the use of surrogate motherhood, which is not allowed in the majority of countries. Porcu et al., (2008) reported the first pregnancy and delivery from frozen eggs in a cancer patient. After freezing her oocytes, the patient underwent bilateral ovariectomy for ovarian cancer and four years later conceived using her own cryopreserved oocytes, and carried on the pregnancy herself. Seven oocytes were retrieved before ovariectomy. After four years three oocytes were thawed. The survival rate of 100% as well as the fertilization and cleavage rate of 100% demonstrated that eggs may be safely stored for several years. The twin pregnancy progressed uneventfully to term with the birth of healthy twins. The duration of oocyte storage does not seem to interfere with the oocytes’ survival as pregnancies occurred even after several years of gametes cryopreservation in liquid nitrogen.

The same group at the University of Bologna has recently obtained the birth of a healthy male from a frozen oocyte of a breast cancer patient. The patient underwent oocyte cryopreservation before chemotherapy for breast cancer. Sixteen oocytes were collected and 14 cryopreserved. After two years and seven months, a cycle of oocyte thawing was performed, with the thawing of four oocytes, all of which survived and were fertilized. Three embryos were transferred into the uterus leading to a single pregnancy that led to the birth of a healthy male at 37 weeks’ gestation.

The correct selection of candidates for fertility preservation by oocyte cryopreservation is very important, in order to offer the most suitable and safe technique to each patient. Oocyte cryopreservation is, actually, the best technique for women without a partner, because it does not require surgery and it has already resulted in a significant number of live births, unlike ovarian tissue cryopreservation, which is still an experimental technique. The selection of patients should take into account the age of the women and should include an ovarian reserve assessment by non-invasive methods, such as anti-Mullerian hormone (AMH) and follicle-stimulating hormone (FSH) levels and antral follicles count.

A limit of oocyte cryopreservation is the timing of the procedure; in fact it cannot be proposed to women that must immediately begin chemotherapy because it requires 10–15 days for the ovarian stimulation and oocyte retrieval. Another important issue is the choice of the stimulation protocol, which should take into account the type and stage of the neoplastic disease and the receptor assessment. In cases of estrogen-sensitive tumors the stimulation should be adapted in order to minimize the increase in estrogen levels: this can be achieved by reducing the dose of gonadotropins and by adding aromatase inhibitors to gonadotropins. The most recommended aromatase inhibitor is letrozole at the dose of 5 mg/die, because it shows a good tolerability and a high efficacy in reducing oestradiol levels (Azm et al., 2008; Requena et al., 2008).

An interesting application of oocyte cryopreservation in association with transplantation of ovarian cortical tissue in a patient with breast cancer has recently been performed, resulting in the birth of two healthy males. A 36-year-old patient diagnosed with atypical medullar breast cancer underwent a laparoscopic right ovarian cortex extraction before chemotherapy and radiotherapy. The ovarian tissue was cryopreserved using a slow freezing protocol and thawed after two years, when the patient was considered free of disease. The cortical strips were attached to the left ovary that appeared depleted of follicles. Then the patient underwent four different cycles of ovarian stimulation, two of these followed...
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by the vitrification of nine oocytes overall. At warming, all the oocytes survived and seven achieved fertilization; two of these were replaced in the uterus leading to a twin pregnancy (Sánchez-Serrano et al., 2010) (Table 1.3).

An additional strategy to preserve female fertility is oocyte cryopreservation after retrieval of immature oocytes from ovarian tissue, in the absence of previous ovarian stimulation (prior to freezing). This technique can be applied in patients with polycystic ovary syndrome (PCOS), where ovarian stimulation is associated with a significant risk of hyperstimulation syndrome, or in young women without male partners who are affected by malignancies and must begin cytotoxic therapy immediately. The cryopreservation may cover oocytes at the stage of germinal vesicles, or in vitro matured oocytes. In the first case, in vitro maturation (IVM) occurs after the thawing; the cryopreservation of these immature oocytes, which are characterized by widespread chromatin, seems able to prevent depolymerization and, theoretically, reduces the risk of aneuploidy and polyploidy.

Current literature suggests that to obtain a homogeneous cohort of oocytes to mature in vitro, the largest follicular diameter should be 12 mm. Normally the oocytes in this cohort of follicles are all germinal vesicle stage at collection (Smitz et al., 2011). Oocyte maturation is defined as the resumption and completion of the first meiotic division from germinal vesicle stage to metaphase II stage; to achieve competency the fully grown oocyte must undergo nuclear maturation and cytoplasmic differentiation. An efficient IVM protocol must be able to reproduce an environment that will not only sustain oocyte growth but also allow full competence.

The first human oocyte IVM was reported by Edwards (1965). Since this first experiment, the methodology has been refined and considerable progress has been achieved in terms of oocyte maturation and pregnancy rates. In 1988 Mandelbaum obtained, after thawing of frozen oocytes at different maturation stages, the same percentage of morphologically intact oocytes for each maturation stage. In contrast to that observed in guinea pigs, mature human oocyte cryopreservation did not appear clearly correlated with the best morphological characteristics. Sixty percent of immature oocytes resumed meiosis after thawing, and only 8% reached metaphase II (Mandelbaum et al., 1988b). The first successful IVM birth resulted from immature oocytes collected at Cesarean section in an oocyte donation cycle (Cha et al., 1991).

Toth reported an 83.3% (60/72) maturation rate to metaphase II for prophase I oocytes, with a 57.7% fertilization rate and a 3.3% blastocyst rate. According to this study, cryopreserved immature oocytes can resume meiosis after thawing, and reach full maturation, with maturation rates, fertilization and cleavage rate comparable to those of non-cryopreserved oocytes (Toth et al., 1994). This finding was not confirmed in subsequent studies that achieved very low maturation rates and fertilization rates (Son et al., 1996). Park et al. in 1997 sought to provide an explanation for the limited success in terms of fertilization and development, assuming damage to chromosomes and microtubules of immature oocytes. They concluded that in vitro matured oocytes that had previously been cryopreserved showed an increased incidence of chromosomal and microtubular abnormalities (Park et al., 1997). In 1998, Tucker et al. obtained the only birth after germinal vesicle cryopreservation; 3 out of 13 cryopreserved germinal vesicles survived, 2 oocytes had matured and fertilized normally, with the birth of a healthy female (Tucker et al., 1998a).

The overall data published suggest that oocyte cryopreservation should be performed at the mature metaphase II stage, following IVM, rather than at the immature germinal
## Table 1.3. Clinical results achieved with oocyte cryopreservation in oncological patients

<table>
<thead>
<tr>
<th>Patient’s age</th>
<th>Pathology</th>
<th>Oocytes retrieved (n)</th>
<th>Freezing protocol</th>
<th>Oocytes cryopreserved (n)</th>
<th>Oocytes thawed (n)</th>
<th>Oocytes survived (n)</th>
<th>Oocytes fertilized (n)</th>
<th>Embryos transferred (n)</th>
<th>Clinical pregnancy (n)</th>
<th>Live birth (n)</th>
<th>Mode of delivery</th>
<th>Weight</th>
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<tbody>
<tr>
<td>27</td>
<td>Hodgkin’s lymphoma</td>
<td>13</td>
<td>Slow freezing</td>
<td>12</td>
<td>1(^{\circ}) cycle: 5</td>
<td>3</td>
<td>3</td>
<td>1(^{\circ}) cycle: 4</td>
<td>Yes (twin pregnancy)</td>
<td>1 (healthy male at 37 weeks’ gestation)</td>
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<tr>
<td>26</td>
<td>Ovarian cancer</td>
<td>7</td>
<td>Slow freezing</td>
<td>7</td>
<td>2(^{\circ}) cycle: 4</td>
<td>3</td>
<td>3</td>
<td>3(^{\circ}) cycle: 3</td>
<td>Yes (twin pregnancy)</td>
<td>2 (healthy females at 38 weeks’ gestation)</td>
<td></td>
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<tr>
<td>38</td>
<td>(papillary serous</td>
<td>9</td>
<td>Vitrification (after transplantation of ovarian tissue)</td>
<td>9</td>
<td>1(^{\circ}) cycle: 5</td>
<td>3</td>
<td>7</td>
<td>1(^{\circ}) cycle: 4</td>
<td>Yes (twin pregnancy)</td>
<td>2 (healthy boys at 33 weeks’ gestation)</td>
<td></td>
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<tr>
<td>37</td>
<td>carcinoma of borderline</td>
<td>16</td>
<td>Slow freezing</td>
<td>14</td>
<td>2(^{\circ}) cycle: 4</td>
<td>3</td>
<td>3</td>
<td>1(^{\circ}) cycle: 3</td>
<td>Yes</td>
<td>Cesarean section mild gestosis</td>
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<td></td>
<td>malignancy)</td>
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- **Porcu, 2008 (first birth in patient with ovarian cancer)**
- **Sánchez-Serrano, 2010 (birth in breast cancer after transplantation of ovarian tissue)**
- **Porcu, unpublished data, 2012 (birth in patient with breast cancer)**

### Notes:

- **Mode of delivery**
  - Elective Cesarean section
  - Cesarean section mild gestosis

### Weight
- 3062 g
- 2100 g, 2400 g
- 1650 g, 1830 g
- 3080 g
vesicle stage, because the potential of oocyte maturation is reduced when immature oocytes are cryopreserved (Cao and Chian, 2009).

Recently, several studies have been carried out to investigate oocyte vitrification after IVM of immature oocytes. In 2007 Huang reported the successful vitrification of three in vitro matured oocytes as a strategy of fertility preservation in women with borderline ovarian malignancy (Huang et al., 2007a). The first live birth after vitrification of in vitro matured oocytes is due to Chian et al. (2009), who achieved the birth of a healthy baby weighing 3480 g at term gestation following the recovery of 18 oocytes at the germinal vesicle stage. Sixteen out of 18 oocytes reached maturity and were vitrified; 4 oocyte survived after thawing, enabling the transfer of 3 embryos (Chian et al., 2009).

Another strategy for fertility preservation is the use of immature oocytes harvested from ovarian biopsy specimens and vitrified following IVM. This technique has been applied by Huang et al. (2008b) in four cancer patients, achieving a mean maturation rate of 79%. In total, eight oocytes were vitrified (Huang et al., 2008b).

A new approach to the freezing of immature oocytes for preventing infertility provides the ability to recover oocytes from the luteal phase of the menstrual cycle and following IVM (Demirtas et al., 2008; Oktay et al., 2008). The rationale for this intervention is the need to shorten the waiting time before antineoplastic treatment.

On the whole, IVM associated with cryopreservation of oocytes is a promising fertility preservation strategy for women who cannot undergo stimulation cycle for IVF, because it allows the complete avoidance of the potentially negative effects of drugs used in the ovarian stimulation and enables the freezing of oocytes within a short time period, so that the anticancer treatment can be started as soon as possible.

Despite these good assumptions and the growing number of IVM/oocyte cryopreservation cycles (the McGill Reproductive Centre has applied the technique in 70 patients with malignancies) (Ara et al., 2010), we do not have actual clinical results in terms of pregnancies and live births in patients with malignancies that can be used to confirm the efficiency of the technique. Moreover, overall pregnancy rates in the other groups of patients (mainly patients at high risk of ovarian hyperstimulation syndrome [OHSS]) remain lower than those achieved in IVF cycles, suggesting the need for further development of the IVM/cryopreservation technique before it is included among the reliable options for fertility preservation.

The same technique of oocyte cryopreservation allowed the vitrification of eight oocytes to save fertility in a patient with Turner’s syndrome (Huang et al., 2008a), a genetic pathology that affects about 50/100 000 females’ live births. Turner’s syndrome is characterized by highly variable genetic anomalies that consist in a partial or complete deletion of the X sexual chromosome; it can be present as a monosomy or as a mosaicism with two or three different cellular lines. Fifty percent of Turner’s syndrome patients have a 45 X0 karyotype, while the remaining cases have karyotypes with mosaicism or X isochromosome or with partial or whole Y chromosomes. Turner’s syndrome is classified among the conditions of premature ovarian failure (POF). The number of germinal cells is normal until 18 weeks of gestation; then the degeneration process begins. From early childhood (2–5 years) increased levels of FSH and luteinizing hormone (LH) are detected and in the adult age these reach menopausal levels.

Up to 30% of patients with Turner’s syndrome show sign of pubertal development and 2–5% present regular menstrual cycles without therapy. Two percent of these patients have a spontaneous pregnancy (Hjerrild et al., 2008). Recently, some ovarian follicles have also