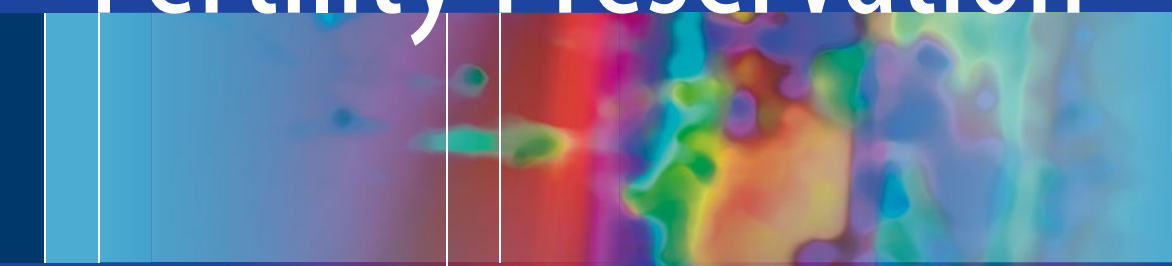
Gonadal Tissue Cryopreservation in Fertility Preservation



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Preface

In young females, treatment of cancer can cause gonadal dysfunction, loss of fertility, and premature menopause. Cryopreservation of gametes and/or embryos and displacement or shielding of the ovaries during radiation therapy are the standard methods for preserving the fertility of young female cancer patients. In 2004, Professor Donnez reported achievement of the first live birth after ovarian tissue cryopreservation and transplantation. Subsequently, ovarian tissue cryopreservation and transplantation has come to be applied clinically as a new option for fertility preservation. In Europe and the United States, a new field named oncofertility has been established to revitalize the medical approaches to fertility preservation in young cancer patients.

It is anticipated that there will be further progress in fertility preservation techniques for young patients with cancer. Progress in fertility preservation is linked to the improved survivorship of young cancer patients and increases options for patients who wish to preserve their fertility. Development of optimum methods for fertility preservation will allow young cancer patients to concentrate on treating their disease. It is more essential than ever to provide patients who require gonadotoxic therapy with information about the risk of loss of fertility and the techniques that are available for fertility preservation. Accordingly, healthcare providers need to keep up with the latest information on fertility preservation, since rapid progress is occurring in this field. When Professor Donnez reported a successful live birth after transplantation of cryopreserved ovarian tissue, it was a breakthrough for both fertility preservation in young cancer patients and research into reproductive medicine. It is now 12 years since that report, and this book, *Gonadal Tissue Cryopreservation and Fertility*, is being published to provide young cancer patients in Asian countries, where oncofertility has attracted increasing attention in recent years, with the latest information in regard to fertility preservation. I would like to express my deepest gratitude to Professor Donnez, who served as a coeditor of this book. He has been my mentor and has provided willing cooperation during the publication process. I also express my heartfelt thanks to Ms. Makie Kambara and Ms. Kanako Honma at Springer Japan KK,

who gave me the precious opportunity to publish this book. I sincerely hope that young cancer patients can beat their disease and that we can achieve a better quality of life for the survivors.

Kawasaki, Kanagawa, Japan Nao Suzuki

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Chapter 1
Oocyte Cryopreservation

Javier Domingo, Ana Cobo, and Antonio Pellicer

Abstract Fertility preservation has become an emerging discipline for any patient whose reproductive function is threatened. Oocyte vitrification is an established method that provides an excellent clinical outcome. It has become an important part of cancer treatment, but also for other non-oncological reasons, with age or the delay of motherhood as the most frequent reasons nowadays for patients to vitrify their oocytes in order to avoid the age-related infertility. Oocyte vitrification is also useful in different gynecological situations in the clinical practice in assisted reproductive technology (ART) where the delayed embryo transfer should be recommended, such as high risk of hyperstimulation syndrome, bleeding, or the presence of hydrosalpinx or polyps. Clinical pregnancy rates in both cancer and social indications are similar to those observed in conventional IVF treatments, with no increase in adverse obstetric and perinatal outcomes in children conceived from vitrified oocytes or embryos. But there are some limitations that must be known: limited number of IVF cycles depending on the number of vitrified MII oocytes, and survival rates after warming or the outcome of IVF with vitrified oocytes are highly dependent on maternal age at the time of freezing.

Keywords Oocyte • Cryopreservation • Vitrification • Fertility preservation

1.1 Introduction

Oocyte cryopreservation has entailed important advantages for human IVF as advances in cryopreservation methodologies have dramatically improved the efficiency of oocyte cryopreservation in the last few years. Vitrification has proven to

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be a very useful tool for oocyte cryopreservation, becoming a great option for a variety of patients.

Advances in oncological treatments and better screening programs have significantly improved survival rates for young patients suffering from different malignancies [1, 2]. These increasing survival rates have encouraged us to focus on the irreversible consequences of chemotherapy, which become more relevant, and have led to increase the number of patients demanding oocyte vitrification prior to chemotherapy during the last years. However, these procedures should not be limited to patients undergoing cancer therapies, but also applied to any situations where the reproductive function is threatened as some other non-oncological conditions [3], and even more oocyte cryopreservation could meet the expectations of women wishing to delay childbearing for a variety of reasons, simplify the egg donation programs, or just offer a less ethically disputable alternative to embryo cryopreservation [3, 4].

Therefore, since fertility preservation procedures showed benefit for medical or nonmedical patients, a new field in assisted reproductive technology (ART) arose and a new population appeared in fertility clinics.

Moreover, the habitual strategy in assisted reproduction has changed considerably and has given solutions to different clinical situations as unexpected lack of spermatozoa, the presence of polyps or hydrosalpinx during the ovarian stimulation, or high risk of hyperstimulation syndrome in which oocyte cryopreservation can be conducted [5, 6].

Fertility preservation should be considered for oncological patients since the moment the diagnosis is confirmed. At this respect several strategies have been proposed to protect or preserve the ovarian function in patients undergoing chemotherapy [7]. Some of them have demonstrated their efficiency, while others are under evaluation or still need to be improved [8, 9].

Immature egg retrieval for further in vitro maturation (IVM) and vitrification, although of great interest in the future, still does not offer feasible options to the patients [10]. There is no doubt that it will have an important role in the future, as trend on fertility preservation techniques is directed to the combination of ovarian tissue cryopreservation as a source of follicles for further retrieval of immature oocytes and in vitro maturation and later vitrification subsequently, which would make avoidance of the ovarian stimulation or the delay on the initiation of chemotherapy possible.

Oocytes may be cryopreserved at any stage, but mature or germinal vesicle (GV) stage oocytes for later IVM are preferred, although more efficacy has been observed when those GV are matured first with vitrification only for those reaching maturity instead of vitrifying GV and then matured in vitro after warming [10].

1.2 Oocyte Vitrification

Vitrification has nowadays completely replaced the conventional technique of slow freezing, since results observed with this approach are significantly lower, and though it is considered the current method of choice to cryopreserve human oocytes and blastocysts, providing an excellent clinical outcome [11]. Oocyte vitrification is an established method, no longer considered experimental [8] as it keeps developmental competence after warming and has been proven to provide consistent success, with high survival rates and pregnancy and implantation rates similar to those obtained with fresh oocytes [12]. Furthermore, the approach is simple and feasible and, regarding the outcomes achieved, effective and consistent.

Historically, the slow-cooling method for oocyte cryopreservation was hampered due to its low efficiency and because it did not guarantee reproducible results. Nearly 100 frozen eggs were needed to achieve a live birth, demonstrating the difficulty and lack of progress performed. However, in later years, the success rate increased due to a better understanding of oocyte physiology, the use of improved media, and of course the leap came when vitrification was incorporated [13].

Vitrification consists in solidification of a solution by extreme elevation of viscosity using high cooling rates from −15,000 to −30,000 °C/min, avoiding the transition temperature for the crystallization of the solution and preventing the formation of intracellular ice crystals, and thus the damage and the osmotic effects caused by ice formation [14]. The real relevance of the oocyte vitrification approach is that it is able to provide the opportunity of postpone childbearing to whenever the patient wishes, or the time she is cured from the cancer that motivated oocyte vitrification, allowing similar IVF cycle prognosis to the moment of vitrification but a few years later. The developmental capability of embryos obtained from vitrified oocytes is maintained with the use of this technique, with no significant differences observed in fertilization rates, embryo cleavage, or clinical results to those achieved with fresh oocytes [11, 12].

Contribution of vitrification has been high, although there are some potential drawbacks associated, especially with the first protocols applied. One of these problems is the toxicity inherent to the use of cryoprotectants which can be reduced by the use of an adequate combination of cryoprotectants (ethylene glycol + dimethyl sulfoxide (DMSO) + sucrose) [15] or by using very low volumes when loading the samples, increasing considerably the cooling rate and allowing to the reduction of the cryoprotectant concentration used [13].

Several storage systems have been designed. Carriers for loading and storing oocytes, all minimal volume containers, can be “open or closed systems” (open or closed pulled straws, Cryoloop, Cryotop, Cryolock, Hemi-Straw system, Cryolife, CryoTip, Fibre Plug, etc.). Close systems prevent the direct contact with liquid nitrogen, thus avoiding the hypothetical risk of cross-contamination, but greatly slow the cooling rate compromising survival rates as a result. However, it should be mentioned that closed systems provide efficient survival rates and clinical outcomes for early cleavage and blastocyst stage embryos. Anyway, so far the most preferred

method for oocyte vitrification is the open system due to its ability to provide highly consistent efficient survival rates and clinical outcomes.

The Cryotop method is a minimal volume device where oocytes are vitrified in volumes lower than 0.1 μl, preserving their capacity for fertilization and further development after warming [13]. In our experience at IVI group with the Cryotop method, survival rates of 97 % have been referred in young patients, with no differences in fecundation and implantation rates, embryo quality, or clinical results when compared to those achieved with fresh oocytes [12, 16]. With this device, the final volume is ten times less than other systems that use minimum volume; therefore freezing rates of –23,000 °C/min are achieved, and moreover, the total concentration of cryoprotectants is reduced to 30 %. Another advantage of the Cryotop method is that it will allow warming rates of 43,000 °C/min, higher to those achieved with other techniques, with ice formation being virtually impossible, which completely eliminates freezing damage and definitely excludes the risk of the zona pellucida being fractured [13].

The risk of cross-contamination has been a point of concern for open systems. Cross-contamination has been related to liquid nitrogen tanks in experimental conditions [17] but never has been reported related to ART cryotransfers. Oocyte cryopreservation procedures have to be efficient and consistent but also safe, and to prevent the risk associated with the direct contact with liquid nitrogen during storage, different strategies have been proposed, as the sterilization of the liquid nitrogen by filtering or by ultraviolet irradiation [18] or the use of vapor phase storage tanks that guarantee optimal conditions during storage at the same temperature of the liquid nitrogen but without being in contact with it and no negative effect on survival and outcome of those vitrified oocytes [19].

Another point of concern about its safety has been the possibility of increasing aneuploidies in the embryos resulting from warmed oocytes, and as a cryopreservation technique, the oocyte vitrification process has to maintain also the structural and genetic integrity. For many years, it has been suggested that the impaired potential of thawed oocytes could be related to the meiotic spindle's high sensitivity to cryopreservation, which may cause an increase in aneuploidy rates affecting the resulting embryos. Different publications showed no abnormal or stray chromosomes from previous frozen oocytes [20] and no differences in the rate of embryonic aneuploidies between embryos from fresh or temporarily vitrified oocytes from the same cohort after microarray analysis [21]. As a result, no significant increase in abnormalities in pregnancies derived from cryostored oocytes has been reported [21, 22]. These results indicate that the chromosome segregation during the anaphase is not impaired by the vitrification process or that it has the capability of restoration. Similar results were found irrespective of different vitrification protocols used, with similar normal spindle configuration between fresh and vitrified oocytes showing that the MII spindle returns to its normal configuration after 3 h of post-thawing incubation under standard conditions [23].

1.3 Technical Aspects of Oocyte Vitrification

The capability of preventing ice formation during all phases of the vitrification process (cooling, exposure to the cryoprotectants, storage, or warming) will condition the feasibility of the oocyte. Crystallization is not compatible with any living organism. There are several factors depending on the oocyte itself that can condition its viability, as its big volume (~150 μm) or its spherical shape, and the content being very rich in water. That gives worse tolerance and hinders the uniform distribution of cryoprotectants, increasing the risk of crystallization. And of course the number of cells is important, and so it can be considered an "all or nothing phenomenon" after warming, as the oocyte is a single cell in comparison with embryos or other tissues.

Another factor that determines the oocyte's viability at cryopreservation is the presence of the meiotic spindle in metaphase II oocytes. Its integrity can be affected by changes of temperature and though the risk of aneuploidies may be increased. Depolymerization of the meiotic spindle occurs at low temperature with tubulin disassembling during the dilution step at warming, although it usually repolymerizes itself after returning to the physiological temperature, reassembling the meiotic spindle. Any deleterious effect of crystallization on the meiotic spindle, which contains the chromosomes aligned, would affect its integrity and consequently its outcome originating aneuploid embryos.

The main potential drawbacks associated with the great cryo-sensibility of oocytes are chilling injury, crystallization, and cryofracture.

Chilling injury mainly associated with slow freezing, may occur at the transition between 15 and –5 °C. This effect on the cells affects the lipids of the membrane and the microtubules of the meiotic spindle and hardens the zona pellucida, being highly vulnerable to low temperatures [24]. This effect can be avoided with a fast transition through this range, with the high cooling rate procedures as provided by vitrification.

Crystallization of the medium surrounding the cells or their cytoplasm is one of the most frequent harmful effects of slow freezing by acting mechanically on the cell structures. During the equilibrium the oocytes respond osmotically to the hypertonic solution of cryoprotectants by means of dehydration. Intracellular ice formation usually occurs when the cell is not sufficiently dehydrated, at the transition between –5 and –80 °C, damaging mechanically the structure of the cell membranes and affecting its viability, especially at thawing [25]. Vitrification is able to virtually avoid crystallization.

Cryofractures affect mainly the zona pellucida, at the range between –50 and –150 °C, due to the mechanical effect of ice crystals [25].

Oocytes suffer an evident shrinkage at dehydration, but gradually re-expand and recover their initial appearance as soon as the cryoprotectant permeates into the cytoplasm. These drastic changes occurring during vitrification may cause

subcellular effects on the oocytes. At warming, it's important to avoid variations in the temperature since Cryotops are taken from the storage bank until they are immersed directly into the plate with the devitrification solution. The temperature of the medium is also very important, which has to be at 37 °C to allow warming rates so high as 43.000 °C/min, and then continue with the dilution solution and removal of cryoprotectants and replacement of cellular fluids prior to washing. In conclusion, the speed of both the vitrification and the warming steps is critical, and any variation of temperature will condition the survival of the vitrified oocytes by increasing the risk of ice crystal formation.

1.4 Clinical Aspects of Oocyte Cryopreservation

Fertility preservation started becoming increasingly important to improve the quality of life in cancer survivors. But fertility preservation should not be limited only to cancer patients but in any situation in which ovarian function is compromised, as other non-oncological diseases or different situations related to ovarian surgery as endometriosis, or just simply women who wish to delay childbearing. A variety of techniques are available for fertility preservation, and they can be used individually or together in the same patient to maximize efficiency.

The ovarian cortex incorporates a finite number of primordial follicles, which will decrease with age due to mechanisms of ovulation and especially for atresia. Fertility potential may be compromised in cases of gonadal removal by surgery, as a consequence of the use of chemotherapy, abdominal or pelvic radiation therapy, or even due to the tumor itself acting on the gonads. Radiotherapy and chemotherapy will, therefore, accelerate the natural decline in the number of follicles [26].

Gonadotoxicity, understood as a reduction of ovarian activity, will depend on various factors, such as age; ovarian reserve; type of chemotherapy, especially the use of alkylating agents; type of cancer; and cumulative doses received [27, 28]. As it is an effect directly related to age and the initial state of gonads, young patients may confer some protective effect since many recover their ovarian function and reproductive capability once chemotherapy is completed, especially with low-dose and low-gonadotoxic chemotherapy [29]. But although many may recover their ovarian function after chemotherapy, egg quality may be suboptimal, so the possibility of pregnancy will maintain decreased. Therefore, options to preserve fertility should be considered from the moment of diagnosis.

Most combination chemotherapy regimens include the alkylating agent cyclophosphamide, which is known to cause a significant loss in ovarian follicle reserve, which may result in infertility and early menopause. Radiotherapy and chemotherapy cause irreversible destruction of germ cells by a direct apoptotic effect on oocytes, with a loss of gonadal hormones and increasing the possibility of germ cell mutagenicity and teratogenic effects [30]. The effect of chemotherapy on the ovary is not an all-or-nothing phenomenon, but can be severe or cumulative with the ovaries having limited recovering capability. This impossibility of regeneration

after damage produced by cancer treatments, which is progressive and irreversible, and ovarian reserve decreasing with age will mark the ovarian response to chemo- and radiotherapy. Women who have been treated with chemo- or radiotherapy may have irregular periods but also infertility and even premature ovarian failure with decreased follicular pool as a consequence of germinal damage. Thus, cumulative dose in adolescents to cause premature ovarian failure is higher than in adult women [26–28].

In conclusion, chemotherapy and radiotherapy have a major impact on reproductive potential, and fertility preservation procedures should be carried out prior to these treatments. The need for fertility preservation has to be weighed against gonadotoxicity related to the type and doses of the chemotherapy the patient will receive [28] and morbidity and mortality associated with cancer itself.

But oncological treatments are not the only causes that lead to ovarian failure. Nowadays, the most frequent reason why patients decide to vitrify their oocytes for fertility preservation is the delay of motherhood, due to different social and economic factors associated with modern lifestyle. The negative effect of age on fertility is well established, especially in women over 35 years of age, for whom the poorer quality of oocytes and the decreasing ovarian reserve are of great concern as will condition future subfertility [31, 32]. In these cases, cryobanking their own oocytes is a good option for women who plan to delay childbearing.

Several strategies have been proposed for fertility preservation, but two are the main techniques usually proposed to patients: oocyte vitrification and ovarian tissue cryopreservation, although not exclusive, each one with its advantages and limitations.

Ovarian tissue cryopreservation may allow patients to achieve pregnancies spontaneously without limitation in time while the graft is viable and incidentally bring the woman some hormonal levels in the event of total loss of ovarian function. Although live births are increasing constantly with this technique, its true effectiveness is discussed as the total number of failed implants is not well known. This reason along with the possibility of reseeding tumor cells into cured patients with the graft makes still considered investigational [33].

Oocyte vitrification should be the elective approach for patients demanding fertility preservation for nonmedical reasons. Oocyte vitrification is an established method no longer considered experimental, with consistent results and whose efficiency has been proven as previously commented, but has its own limitations, as the number of available metaphase II oocytes which can limit the number of IVF attempts and survival rates, mainly related to patient's age and quality of eggs, and more vitrified oocytes are required to achieve equivalent cumulative ongoing pregnancy rates in blastocysts than when early cleavage-stage embryos are transferred [34].

Specialists, patients, and their families should be aware that fertility preservation can be considered and reproductive function preserved in any condition where fertility can be threatened. A multidisciplinary collaboration in the management of these patients between oncologists, gynecologists, surgeons, hematologists, pediatricians, and reproductive specialists is needed to improve awareness and

availability. Decisions should be made individually, both when recommending fertility preservation or allow attempting a pregnancy.

1.4.1 Clinical Indications

The usefulness of this strategy becomes very clear for those women who need an option for fertility preservation, like patients diagnosed with malignant diseases receiving chemo or radiotherapy and that will suffer from gonadal failure and infertility after their cancer treatment or other non-oncological conditions such as autoimmune disorders that need to be treated with chemotherapy. Women who postpone conception until late reproductive years, recurrent endometriosis, or some chromosomal abnormalities that can lead to ovarian failure can also benefit of this fertility preservation procedure [3, 8, 35].

Cryopreservation of the female gametes has represented an important challenge since the beginning of ART given its potential for overcoming several of the problems that arise during fertility treatment, such as people with high risk of hyperstimulation syndrome, appearance of an hydrosalpinx or polyps during the stimulation, unexpected absence of spermatozoa the day of the ovum pickup, low-responder patients to accumulate oocytes from two or three stimulation cycles, any bleeding prior to embryo transfer, and ethical concern about embryo cryopreservation [36, 37]. Moreover, the establishment of egg banking system for ovum donation programs would considerably simplify the logistics by which oocytes could be donated since no synchronization between the donor and recipient should be needed, shortening the days of estradiol replacement until donation. Oocyte cryopreservation, similarly to semen banks, would also allow a more accurate screening for viral infections [38].

When planning oocyte cryopreservation for fertility preservation in a cancer patient, always under our criteria with the oncologist's authorization, different factors must be considered, as age, time until chemotherapy will begin, the need of an ovarian stimulation, and whether the tumor is hormone sensitive or not. For those cases where the time hiatus between diagnosis and treatment is not always available or the potential risks associated with high estrogen levels do not recommend an ovarian stimulation, or in the case of girls, ovarian tissue cryopreservation for later autotransplantation should be the first elective approach.

Fertility preservation procedures are not limited to cancer patients but can be applied in other non-oncological situations which may lead to ovarian failure, some of them being medical situations while others nonmedical.

Severe systemic autoimmune diseases should be one of these situations as they may require therapy potentially harmful to their ovaries as cyclophosphamide for refractory rheumatoid arthritis [39], for severe manifestations of systemic lupus erythematosus, such as proliferative nephritis, affection of the central nervous system, pneumonitis or severe thrombocytopenia [40], or in other diseases such as Wegener's granulomatosis [41]. Patients needing bone marrow transplantation

are also associated with high risk of ovarian failure as high doses of chemo- and radiotherapy are applied to destroy the preexisting bone marrow [42].

Repetitive conservative surgery on the ovaries can also lead to premature ovarian failure by diminishing ovarian reserve, and furthermore the pregnancy rate is almost half the rate obtained after primary surgery [43]. Endometriosis plays an important role as one of the most frequent pathologies in gynecologic surgery, although there are other benign surgical procedures that can result in a loss of ovarian function. Excision of endometriotic cysts is associated with a significant reduction in ovarian reserve as normal ovarian tissue is removed and destructed by electrocoagulation, and therefore they only should be removed if pain, infertility, and large size of the endometriomas obstruct the oocyte retrieval at the ovum pickup [44].

In the same manner, patients with mosaic Turner syndrome should be candidates for oocyte vitrification as premature ovarian failure is commonly associated with this chromosomal abnormality [45] or patients with conservative surgery diagnosed with early-stage borderline ovarian tumors as a preventative measure in case of recurrence and adnexectomy [46].

Ovarian hyperstimulation syndrome (OHSS) is one of the most serious iatrogenic complications of IVF, and embryo transfer should never be done in the same cycle in patients at high risk of it, and therefore an effective strategy could be to vitrify the oocytes for a later embryo transfer in another cycle [47]. In oncological patients cryopreserving their oocytes, many of them young and with good ovarian reserve and whose goal is not to get pregnant, it would be advisable to use protocols with gonadotrophin-releasing hormone (GnRH) antagonists and trigger ovulation with a bolus of GnRH agonist to avoid the possibility of causing OHSS [37]. After GnRH agonist triggering, the antagonist is moved and the receptor is directly activated causing the *flare up* effect which is accompanied by the release of gonadotropins [6]. This *flare up* effect is effective in the final oocyte maturation and ovulation. Advantages consist of the possibility to recover a higher number of mature oocytes [48], shortening of the luteal phase, and avoiding the discomfort related to ovarian hyperstimulation at the time OHSS is largely prevented. This is very important if we consider that most of these patients will start chemotherapy immediately after finishing the process. This should be extended to all patients who wish to vitrify their eggs. Recent publications have reported that this agonist trigger and freeze-all strategy does not prevent severe OHSS at all, as some cases have arisen, so this complication has not been completely eliminated with this approach [49, 50].

Accumulation of oocytes from several ovarian stimulation cycles is a valid strategy for low-responder patients, in order to increase the availability of oocytes to reach a similar situation to normoresponders for a further IVF cycle or if preimplantation genetic screening (PGS) is pretended [51]. This approach is associated with a lower drop-out rate, fewer transfer cancelations, higher live birth rate per intention-to-treat patient, more cycles with vitrified embryos, and higher cumulative pregnancy rate, which endorses the treatment as a successful alternative for low-responder patients. Similar outcome was observed among patients over 40 years of age [52].

Social fertility preservation is a new medical and social phenomenon to prevent age-related subfertility, motivated primarily by the search for a social and labor stability or the absence of a stable partner. The patient vitrifies her oocytes with no medical indication for postponement of childbearing, which means a medical intervention which is not associated with a need. They are fertile and healthy women who choose to delay childbearing for various reasons.

There may be an idea of social fertility preservation postponing the first pregnancy with important demographic and economic consequences related to an inverted population pyramid. The age for a first pregnancy has risen nowadays a mean of 2 years over the past 10 years. Regarding this, the mean age for a first pregnancy in our country has increased to 32 years of age [53]. Age is directly related to subfertility, and nowadays the number of patients in the 40s attempting IVF, with dramatically decreased pregnancy rates, is increasing constantly, some of them with the false idea of ART compensating the natural decline of fertility and taking the success of IVF for granted. More than 50 % of the IVF cycles in Europe are done nowadays in patients over 35 years of age [54]. Oocyte vitrification could be a solution for these patients, even more if there is a family history of premature menopause, so this approach should be not so questioned as it was a few years ago, as it would be useful to prevent infertility related to age [55]. Treating older women with their own young oocytes would help to reach better reproductive outcome and diminish the need of egg donation. Female fertility consistently decreases after the middle of the third decade [56], so oocyte cryopreservation should be done ideally earlier.

Oocyte cryopreservation also will be useful to overcome ethical concerns and legal restrictions in several countries associated with embryo cryopreservation.

1.4.2 Clinical Results and Obstetric Outcome: Limitations of the Procedure

The available knowledge of oocytes from ovum donation programs and also on the autologous IVF cycles carried out with vitrified oocytes offer better outcomes in terms of oocyte survival and embryological development of the vitrified and warmed oocyte in comparison with the slow-freezing procedure and similar clinical results when compared with fresh oocytes [11, 57, 58].

In our experience the developmental capability of embryos obtained from vitrified oocytes is not affected by the vitrification procedure, since fertilization, embryo cleavage, quality, and clinical results are similar to those achieved with fresh oocytes [12]. Survival rates so high as 97 % have been referred for young donor patients [12].

The number of pregnancies resulting after an oocyte cryopreservation process is growing constantly, with no apparent increase in adverse obstetric and perinatal outcomes in children conceived with vitrified oocytes as low birth weight or

congenital abnormalities [59, 60]. The incidence of other complications including anemia, diabetes, pregnancy-induced hypertension, preterm premature rupture of membranes, and preterm birth rates is comparable to those observed with fresh oocytes [60].

The availability of egg banking for both ovum donation programs and own oocytes for autologous IVF cycles has proven highly efficient and has conferred remarkable advantages. Outcomes of ongoing data from clinical use of vitrified donor oocytes have confirmed that these procedures are efficient, reliable, safe, and consistent. Cryobanking eggs provides the possibility to match a donor for a recipient from a large donor pool without waiting long for an appropriate match, and donors can carry out their donation cycle independently on the recipient, with no adverse effect on results.

The limitation of the procedure can be given by the number of vitrified MII oocytes that will limit the number of IVF attempts in the future and consequently the availability of embryos and the possibilities of achieving a pregnancy. Age again may condition the results as survival rates decrease with it. Survival of the oocytes is related to the quality of the oocyte what is an age-dependent factor. This lack of survival may serve as a selection filter but overshadows the benefits the procedure could have on low responders, most of them around the 40s. Patients should be advised to ensure a reasonable number of cryopreserved oocytes for which more than one stimulation cycle should be required. The outcome of IVF with vitrified oocytes is, just as for fresh oocytes, highly dependent on maternal age at the time of freezing, but not related to the vitrification process itself [52, 61].

To face the fact of low survival rates in patients where bad oocyte quality may be suspected, embryo cryopreservation instead of oocyte vitrification for differed embryo transfer or just to accumulate for later PGS could be an option as survival rates for them seem to be higher.

Despite oocyte vitrification protocols being simple to perform, experienced hands are needed to guarantee success. A learning curve is necessary to avoid changes in temperature during cooling and warming and results may be influenced until high efficiency is reached.

1.4.3 Ovarian Stimulation

One of the concerns on oocyte cryopreservation is that a prior multiple follicular stimulation is needed as well as a time frame of 2 or 3 weeks, which would delay the beginning of chemotherapy. However, the elapsed time between cancer diagnoses and initiation of treatment may vary among malignancies, especially in breast cancer patients and some leukemias or lymphomas which have to wait 4 or 6 weeks to start the chemotherapy. Another point of concern is whether the stimulation with gonadotropins would affect the evolution of breast cancer, which is the most common malignancy in reproductive age and the most frequent diagnosis of people undergoing any fertility preservation option [62]. High estradiol