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Review Article

Molecular aspects and clinical methods for preserving ovarian reserves in women receiving cancer treatment

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Summary

Cancer prevalence is high, and of importance to cancer sufferers is the long term survival and normal activities resumption. Moreover, pregnancy is drawing interest for preserving ovarian reserves in post-chemotherapy affected women, especially of younger ages. The gonadotoxic effect of cancer treatment, involves mechanisms that are not fully understood, mainly due to the variety of molecular pathways triggered once therapeutic agents applied. Reported rates of premature ovarian failure after the treatment effect and the application of various treatment protocols, differ extensively due to the protocol itself but also due to the age of treated patients. Several options for preserving ovarian reserves are currently employed in the clinique, such as ovarian transposition, embryos cryopreservation and the use of gonadotropin-releasing hormone (GnRH) and its agonists/antagonists, but most of them are still under investigation. This paper reviews these methods and the molecular mechanisms that are possibly involved in the action of agents such as GnRH.

Key words: Ovarian reserves; Molecular; Clinical; Cancer; Treatment.

Introduction

It is estimated that approximately 6 million new cases of cancer, either hematological malignancies or solid tumors, are recorded worldwide every year. About 4 million of them, concern women of all ages and about one million women are survivors of childhood cancer [1].

In developed countries, both screening and use of chemotherapy and radiotherapy, have led to a dramatic reduction in mortality rates of cancer. Additionally, a growing number of women postpone childbearing to older age, due to social or financial causes. It is for such reasons that women younger than the age of 45 years pose a great demand for preserving fertility.

However, cytotoxic therapy is associated with damages to body tissues and cells other than the targeted tumor cells, significant morbidity and long-term physical and psychological effects. Among them, ovarian toxicity is an important and common long-term adverse event of curative chemotherapy and radiotherapy [2]. Since, many of these patients are young, they suffer premature ovarian failure (POF) which annihilates the reproductive function of the ovaries.

This article, reviews the literature, discussing the effects of chemo- and radio-therapy on ovarian toxicity and provides more detail on the possible molecular mechanism for the effect of cancer treatment on female fertility.

Chemotherapy

All chemotherapeutic drugs, have an adverse effect on ovarian tissue, by interrupting vital cell processes and arresting the normal cellular proliferation cycle. Most of the available data for ovarian failure after chemotherapy, is based on leukaemias, lymphomas, Hodgkin's disease, and on some solid tumors such as breast cancer. However, there is an increasing number of patients with no malignancy who are being treated successfully with chemotherapy due to autoimmune diseases, such as systemic erythematosus lupus, rheumatoid arthritis as well as some hematological diseases [1, 3-5].

Chemotherapeutic agents, can be grouped into five classes based on their mode of action: 1) alkylating agents, 2) aneuploidy inducers, 3) topoisomerase II inhibitors, 4) antimetabolites, and 5) radiomimetics [2]. Chemotherapeutic drugs are used as a monotherapy or in combination with other agents in order to increase their anti-tumor effects, but this also leads to an increase in their adverse events. Ovarian damage and failure, is a common long-term side effect of thermotherapy.

The final impact of chemotherapy to ovarian function depends on factors such as the patient's age, the therapeutic protocol, and the dose of the drug administered [1, 6-8].

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Several studies have attempted to clarify the impact of age in determining the effects of chemotherapy on ovarian function. Older women have in general a higher incidence of complete ovarian failure and permanent infertility comparing with younger women [1, 9-11]. These results, can be explained by the larger deposits of follicles that young women bear [12]. Primordial follicles are diminished with age. At puberty, about 300,000 follicles are present and functioning in the ovaries, progressively declining with age to 100 at the time of menopause [12, 13].

In recent years, there is a great interest in new protocols of treatment of all types of cancer. The vast majority of these protocols are based on combinations of chemotherapeutics agents, and results in ovarian failure depend on the agents that are used. Alkylating agents, pose the highest risk in causing ovarian failure [2]. Cyclophosphamide, the most common agent in this category, can cause ovarian fibrosis and also follicular and oocyte depletion. Meirow reports that the substances of this class, are closely associated with the greatest risk among all chemotherapeutics protocols for ovarian dysfunction [14]. Bines *et al.* [15], reviewed reports on ovarian destruction after post-adjuvant chemotherapy applied on premenopausal breast cancer survivors and concluded that the protocols that included cyclophosphamide, have the highest rate of chemotherapy- related amenorrhea.

Cisplatin and its analogs can also cause ovarian failure. Studies with cisplatin treatment in mice, have shown that different types of chromosomal damage are associated with genetic effects in the oocytes which result in early embryonic mortality and aneuploidy [16].

Recently, Li *et al.* and Blumenfeld, have shown that the combination of a gonadotropin-releasing hormone (GnRH) agonist with a GnRH antagonist completely prevented the flare-up effect and enhanced the protective effect of the ovary from cisplatin-induced gonadotoxicity in rats [17, 18]. In addition, agents such as vinca alkaloids can cause aneuploidy. Many experiments in mice have shown that the use of these agents results in malformed fetuses [19]. Anthracycline antibiotics have been implicated in dominant lethal mutations in maturing / preovulatory oocyte in female mice. Etoposide, can also cause aneuploidy in oocytes and pericentric lesions, leading to malformed embryos. The present authors' knowledge up to now, does not allow to draw a safe conclusion regarding the effects of antimetabolites on female germ cell.

Finally, studies have shown that young women treated with chemotherapy agents prior to menarche, exhibited a delay at the start of menstruation, but all had their menarche reappear shortly after cessation of the treatment. Most of young women treated after menarche, developed amenorrhea, while some others treated with very mild drug regimen, had irregular cycles. Data from endocrinological studies, have shown that primary ovarian failure was rare and occurred in adolescent girls only when chemotherapy and radiotherapy were used in combination. In girls with regular menstrual cycles, when treated with high dose of chemotherapeutics agents, an ovulation or inadequate luteal phase could be observed due to hypothalamic effects of stress, anxiety, and emotions associated with the malignant disease [13, 20].

On many occasions, treatment may be shifted from one protocol to another, also taking in effect combined therapies, but for each disease only a few characteristic protocols are commonly used. Therefore, it is very practical to analyze the risk for ovarian failure according to disease type. For example, use of combined chemotherapy in early-stage of Hodgkin's lymphoma can significantly reduce long-term ovarian dysfunction [2].

Radiotherapy

Radiotherapy has adverse events on gonadal function. The degree of the injury depends on factors such as the dose, the irradiation field, and the patient's age with older women, being at a greater risk of damage [1, 2].

Radiotherapy, is used to treat pelvic and abdominal diseases, such as cervical and rectal cancer. It is also used for thyroid cancer, while cranio-spinal radiotherapy is used for central nervous system malignancies. Another application of radiotherapy, is in Hodgkin's lymphoma and other hematological malignancies, when pelvic lymph nodes are influenced by the disease before bone marrow transplantation occurs, when total body is being irradiated.

Previous studies have shown that doses of about 30 Gy used in the case of brain tumors, can cause long-term hypogonadotropic hypogonadism in children [21]. Wallace *et al.* [22, 23], demonstrated that the estimated dose at which half of the follicles are lost in humans (LD50) is four Gy. Every patient exhibits different sensitivity to radiation damage which may be pre-determined genetically, but it seems that the age factor is the most important, as younger women are more likely to preserve their ovarian function, due to the greater primordial follicle reserve [13]. Additionally, where possible, shielding of the ovaries is used, or the radiation field is restricted in order to avoid direct irradiation to the ovaries [5, 24, 25].

Lashbaugh and Casarett [26], indicated in their study that women younger than 40 years of age are less sensitive to ovarian failure after radiotherapy, with an estimated dose of 20 Gy being required to produce permanent ovarian failure, while about six Gy are required for older women. A possible explanation for this is that younger women have better quality follicles, and the cell membrane is more resistant to damages. As the oocyte membrane seems to be among the less sensitive membranes to radiation, older women with poor quality follicles seem to have lost this protective factor.

Chiarelli *et al.* [27], presented in their study the relationship between the risk of premature ovarian failure and the total dose of abdominal-pelvic irradiation, in order to study the long-term effects of radiotherapy in young women. With doses < 20 Gy, the relative risk was 1.02, at dose of

20-35 Gy the risk was 1.37, and at doses > 35 Gy the relative risk of premature ovarian failure was 3.27. The percentage of females who suffered from infertility after radiotherapy, co-related with the patients' age at the time of treatment and was restricted to women who were irradiated after puberty. Also, the percentage of women who suffered infertility, correlated with increasing dosage of radiotherapy: a dose of 20-35 Gy causes 22% rate of infertility and doses > 35 Gy led to a 32% rate of infertility [27].

In addition, Thibaud *et al.*, showed that total body irradiation of < ten Gy given in a single dose before puberty causes a high ovarian failure rate of about 50-80% [28]. On the other hand, Vini *et al.*, concluded that radiotherapy in distant areas like thyroid pose a low risk of permanent damages to the ovaries and these patients can have normal pregnancies after this treatment [29].

Many studies have attempted to show the radiation effect on the uterus and on subsequent pregnancy outcomes [30-32]. Uterine radiation is closely associated with infertility, spontaneous miscarriage mainly in the first trimester, and intrauterine growth retardation [33]. These effects are probably caused by the changes in the uterine musculature and blood flow, as well as from hormone-resistant endometrial insufficiency caused by radiotherapy. A review by Critchley and Wallace, suggests that steroid hormone replacement therapy can be used to improve uterine damage after irradiation, but only in young women. Moreover, it is also known that there is a close relationship between radiotherapy and obstetric complications. Patients who have received radiation treatment in childhood or puberty, show a higher rate of complications such as spontaneous abortions, preterm labor, and low-birthweight infants when compared to the general population, but there is no study to prove the relationship between radiation and teratogenicity [34-39].

In conclusion Langan *et al.*, report that younger women at treatment with chemotherapy were associated with a higher frequency of normal ovarian function post-treatment, whereas adding total body radiation to the regime was associated with a high risk of ovarian failure [40].

Molecular aspects of chemotherapy and radiotherapy effect on the ovaries

Chemotherapy and radiotherapy direct effect

Chemotherapy and radiotherapy constitute the main two therapeutic regimes either as standalone approaches or in combination with other non chemo- or radio- therapeutic agents (e.g. monoclonal antibodies) in order to treat cancer [41, 42]. There is still a necessity for extensive work to be performed on dissecting the molecular events that are triggered in the ovary after different therapeutic regimes have been applied to the patient.

In general though, the main feature of both these therapy regimes is that they induce tissue and subsequent cell and DNA damage [43, 44]. Both of these regimes have also been shown to exert effects on reproduction aspects of the ovary such as the ovarian reserve and fertility.

One example of a classic chemotherapeutic agents is cisplatin. Cisplatin, once introduced into the cells via the assistance of a copper transporter (CTR1), is activated via a series of reactions in which one of the chloride ligands is displaced by water in a slow manner. The complex of cisplatin and water that is formed eventually binds DNA exhibiting a predilection for nucleophilic N7 sites on purines on the nucleic acid chain [45].

At first, the formation of monoadducts is observed. These monoadducts take part in reactions that lead to the formation of inter-strand and also intra-strand crosslinks. The cytotoxic effects of such platinum agents depend on the formation of these crosslinks. Cisplatin's cytotoxic effects may also depend on the formation of adducts that cause conformational changes in the DNA chain, that leads to impairment of separation of the two DNA strands thus impairing the DNA replication and synthesis [46]. These lesions upon formation are able to be recognized by DNA proteins that trigger DNA damage repair and/or apoptosis signaling and the platinum caused cell death is mediated by cell cycle arrest that occurs in the G2 phase of the mitotic cycle [47]. DNA repair includes the nucleotide excision repair pathway (NER) [48, 49], the DNA mismatch repair mechanism (MMR) [50, 51], and the homologous recombination repair pathway [52]. Other chemotherapeutic agents include cyclophosphamide, doxorubicin, melphalan, busulphan etc, that act in various ways in achieving their effect on the target cells [53-55]. Cyclophosphamides and alkylating agents cause dose-dependent destruction of oocytes and also follicular depletion [56-58].

In the case of the ovaries, the gonadotoxic effect of various chemotherapeutic agents such as cisplatin, usually affects oocytes, granulose, and theca cells in a way that may be detrimental to the ovarian reserve. Agents such as cyclophosphamide and melfalan may pose a risk to gamete formation, they are not cell cycle specific, and may cause damage even to resting oocytes [55, 59, 60]. As mentioned above, age seems to be a critical factor deciding the extent of the oocyte and chemotherapy induced ovarian failure with older women posing a greater risk group.

In terms of radiotherapy it is useful to mention that the ovarian follicles are very sensitive and vulnerable to DNA damage caused by ionizing radiation. At the same time oocytes exhibit a rapid onset of events such as chromosome condensation and disruption of the nuclear envelope [61, 62]. There is still a necessity for further research in identifying the molecular cascades triggered by radiotherapy.

Mechanisms of action for ovarian protection

1. GnRH molecular mechanism

Gonadotropins are proteins that were first introduced in the 1960s and have since been used in ovarian stimulation cycles, in order to induce multiple follicular development.

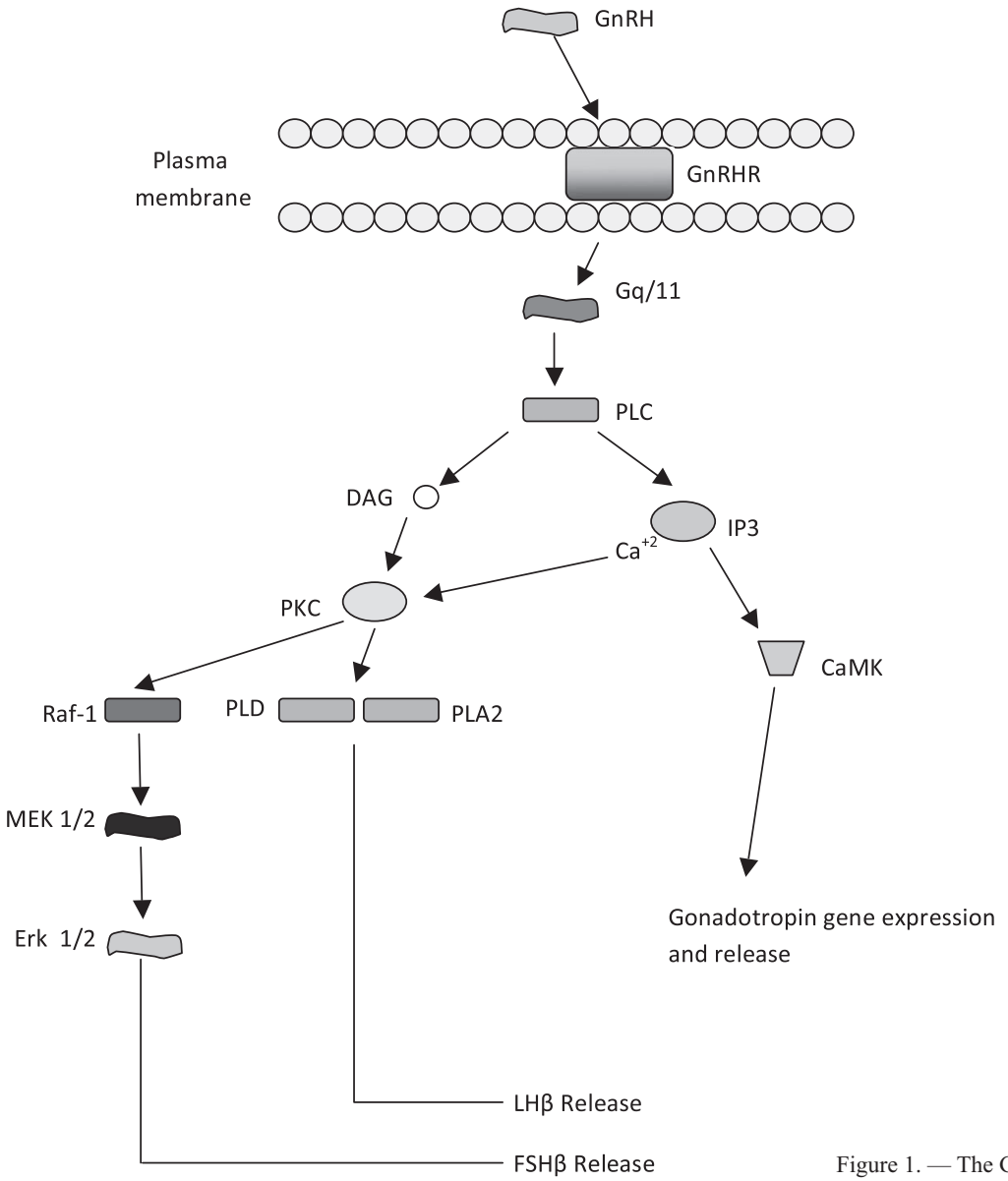

Figure 1. — The GnRH activating pathways.

The GnRH, is a decapeptide and a member of the GnRH family of proteins. It is a so-called trophic peptide hormone that is responsible for the release of the follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary [62, 63]. The protein itself is synthesized and released from the hypothalamus.

GnRH binds to its cognate receptor GnRHR that is located in the anterior pituitary. GnRHR belongs to the family of G-coupled receptors [64]. The binding of the GnRH to its receptor triggers the coupling of the receptor to Gq/11 proteins leading to activation of phospholipase C which transmits its signal to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3) [65, 66].

Following its activation, DAG activates the intracellular protein kinase C (PKC) pathway. IP3, in turn, stimulates the release of intracellular calcium. Other molecules such as phospholipase D, phospholipase A2 (PLA2), and proteins of the MAP kinase signaling pathway are activated that in turn regulate the expression and secretion of molecules such as FSH and LH [66]. These pathways also stimulate the expression of gonadotropin and they are shown in Figure 1.

In later studies GnRH agonists and antagonists have been employed in order to impair follicle depletion. In humans there are studies that have exhibited a reduction in the rate of amenorrhea in over 50% of the patients that underwent treatment with GnRH agonists compared to controls [67, 68]. GnRH agonists bind onto the GnRH receptor and enhance signaling events via the GnRHR thus increasing production of LH and FSH. They are molecules synthetically

modeled after the GnRH decapeptide that carry changes in positions within the ten amino acid chain, especially in positions 6 and 10 [69]. The usual advantage of these molecules is their slower degradation compared to the original GnRH molecule [70, 71].

The GnRH is believed to play a protective role in the case of ovarian reserves in patients that are treated for cancer via the use of chemotherapy and radiotherapy. The pathways that are activated upon binding of GnRH onto its receptor are shown in this figure. Guanine nucleotide binding protein alpha 11 (Gq/11), phospholipase C (PLC), phospholipase D (PLD), phospholipase A2 (PLA2), diacylglycerol (DAG), inositol 1,4,5-triphosphate (IP3), calcium/calmodulin-dependent protein kinase II alpha (CaMK), protein kinase C (PKC), v-raf-1 murine leukemia viral oncogene homolog 1 (Raf-1), mitogen-activated protein kinase kinase 1 (MEK 1/2), extracellular signal-regulated kinase 1/2 (Erk 1/2), LHβ polypeptide (LHβ), FSH, beta polypeptide (FSHβ).

In general, further work is necessary to shed more light into whether GnRH and analogues may actually protect the ovary as studies seem to be contradictory with some of them, suggesting no actual protective effect of these molecules [72]. A more clinical review on the actions of GnRH and its analogues will be considered later.

2. Other mechanisms for ovarian follicles apoptosis and apoptosis inhibition

The actual mechanisms by which damage may occur in the ovaries and the relevant biochemical pathways that are triggered after chemotherapy or radiotherapy are still under investigation. Nevertheless recent experimental data has been indicating that various molecules may play significant roles in preventing ovarian follicle premature death and even more, apoptosis due to cancer treatment.

GnRH may act directly onto its receptor GnRHR in order to inhibit follicle apoptosis [73]. GnRH may also be responsible for the upregulation of a protein called sphingosine-1-phosphate (S1P) [74, 75], thus S1P playing an important role as an apoptosis inhibitor. In turn, another protein, acid sphingomyelinase is the enzyme required to produce ceramide, an early messenger of apoptosis in response to stress [75]. It has been shown in experimental, in vivo, mouse models treated with agents, such as doxorubicin, that the lack of acid sphingomyelinase from their oocytes or when wild type mice were treated with S1P did resist oocyte apoptosis [76]. SIP molecules bind onto receptors termed Edg receptors or S1P receptors [77]. Upon binding onto their cognate receptors they elicit intracellular signalling pathways via regulation of diverse G coupled proteins, exerting their effects [76, 77]

Caspases comprise another family of proteins that may play significant roles in oocytes' apoptosis due to chemotherapy. In mice, it has been shown that in the case of female germ cells and upon an insult in their metabolic status, caspase-2 and caspase-3 molecules are activated and execute the apoptotic signaling cascade. However in the case where DNA damage chemotherapeutic agents are used, caspase signaling pathways are triggered that may involve caspase-12 especially in the case when caspase-2 and caspase-3 are absent [78]. It is also known that caspase-9 may play a role in mouse oocytes´ apoptosis during the meiotic prophase progression [79] but it is still unknown whether it plays a role in the apoptosis of damaged oocytes during chemotherapy.

Except the caspases and SP1 signalling cascades, evidence begins to appear that other molecular pathways may be involved in apoptosis due to chemotherapy. Recent data suggests that platinum damaged oocytes may be rescued via the inactivation of the p53 signalling network [80]. In addition in mice, it has been shown that the thyroid hormone 3,5,3'-triiodothyronine (T3) protects granulosa cells from chemotherapy induced apoptosis [81]. These new discoveries show that there is still much to be considered in terms of the molecular machinery behind oocytes chemotherapy induced apoptosis.

Fertility preservation options for women after cancer

In literature, there are several options for fertility preservation targeted at women after cancer therapy. The use of a fertility preservation method needs individualization and depends on the time of cancer treatment (radiotherapy or chemotherapy), time available, type of cancer, and patient's age.

Apoptotic inhibitors

The general notion on apoptotic inhibitors is that they constitute molecules that are equipping the doctor's arsenal in combating causes for ovarian failure and more specifically assist in reducing the damage in ovarian reserves caused by standard chemotherapeutic agents and radiotherapy [82, 83].

Although great progress has been achieved there, is still a necessity for further research to take place in order for targeted action of apoptotic inhibitors to occur. More specifically, further studies are necessary in order to clarify the negative effect on reducing the tumor mass by a concomitant administration of apoptotic inhibitors and cancer therapeutics such as chemotherapy agents, especially at the molecular level. This process may identify novel targets for designing novel anti-apoptotic compounds and also will provide clinicians with more tools in order to fight tumor more effectively without leading oocytes into cell death.

Ovarian suppression (GnRH analogue treatment)

Many studies have evaluated the utility of treatment with GnRH analogues, in order to preserve ovarian function during cytotoxic therapy. Investigators attempted to render the germinal epithelium quiescent by suppression of go-

nadotropins (using GnRH agonist). This search has suggested that receiving GnRH analogues during radiotherapy and/or chemotherapy, may increase the possibility of a woman to maintain her menstruation after therapy [84, 85].

However, conflicting outcomes on the results of GnRH analogues have been presented, intensifying the debate regarding the existence of FSH receptors in the primordial follicles and GnRH analog receptors in the human ovary [85]. The study of Meirow *et al.*, failed to demonstrate a protective effect of GnRH after chemoradiotherapy in patients undergoing bone marrow transplantation [86]. However, in a recent study Li *et al.*, suggest that the combination of GnRH agonists and antagonists protects primordial ovarian follicles in rats [17] but, this remains to be proved in humans. Three other small randomized trials performed in humans, reported that GnRH analogues was not effective in preserving fertility in patients receiving chemotherapy for Hodgkin lymphoma [87, 88] or breast cancer [89]. Ovarian control markers were not different in the control subjects despite the level rates of amenorrhea in the group receiving GnRH analogs.

The treatment with GnRH analogs should begin at least ten days before the first chemotherapy, due to the initial flare-up effect which causes undesirable ovarian stimulation. Administration should continue until the end of chemotherapy, so that the downregulating effect remains for at least two weeks after the end of treatment. However, no safe results can be obtained by the use of GnRH analogs as the available studies are limited by the small sample size, lack of randomized control group, and lack of definitive information regarding actual fertility outcome [1, 90].

Alternative ways for preservation of ovarian reserve

1. Ovarian transposition (oophoropexy)

This method is suitable for patients undergoing gonadotoxic radiotherapy. The ovarian follicles are sensitive to DNA damage from radiation as the exposure can cause atrophy and decreased follicle number. The degree of ovarian damage, depends on the dose of radiation, the patient's age, and the combination of radiotherapy with chemotherapy [90]. The most common indications for ovarian transposition are cervical and vaginal cancer, pelvic sarcomas, and Hodgkin's disease.

This method is suitable for patients undergoing gonadtoxic radiotherapy alone without chemotherapy. Transposition can be performed laparoscopically just before the start of radiotherapy. Beginning radiation therapy immediately, decreases the chance of failure from ovarian migration back to the field of treatment [91]. The success rate of this procedure varies between 16% and 90% [92, 93]. The failure of this method and the subsequent low rate of success, is due to variant factors such as scatter radiation, dose, patient's age, vascular compromise, and whether ovaries are shielded during the procedure [90]. Complications are relative rare and include chronic abdominal pain, infraction of the fallopian tube, and ovarian cysts [94-96].

2. Cryopreservation

Cryopreservation of embryos, is a proven effective method for preserving fertility. Cryopreservation of oocytes and ovarian tissue are promising approaches, but remain under investigation [97]. Successful cryopreservation of an intact whole human ovary has not yet been successful.

- Cryopreservation of embryos

To date, the most effective approach with regards to fertility preservation is embryo cryopreservation. The human embryo, is resistant to damage caused by cryopreservation procedures. The post-thaw survival rates of embryos are between 35-90% while implantation rates are between 8% and 30%. In case that multiple embryos are available for cryopreservation the pregnancy rate can reach up to 60% and delivery rates per embryo are about 18-20% [98].

- Cryopreservation of oocytes

I. Cryopreservation of mature oocytes (after gonadotropin stimulation).

Cryopreservation of oocytes, is more problematic than sperm or embryo cryopreservation. The main obstacle is the sensitivity of oocyte to chilling, probably because of the sensitivity the spindle apparatus and the higher lipid content of the cells. Cooling and exposure to cryoprotective agents (CPAs) may increase the incidence of aneuploidity in human oocyte due to damages in the cytoskeleton [99]. The eligibility of a woman for this method depends on the type of cancer and woman's age, as a lot of patients may not have more than one opportunity for oocyte harvesting before undergoing chemotherapy or radiotherapy, since one cycle of controlled stimulation requires a few weeks. The success of the method is also dependent on the total number of oocytes harvested as < ten oocytes means very low chances of pregnancy [1].

II. Cryopreservation of immature oocytes after in vitro maturation (IVM) – (without gonadotropin stimulation).

Immature oocytes have been harvested both in situ and excised ovarian tissue [100]. The oocytes can be matured in vitro either before freezing or after thawing. These oocytes are expected to be more resistant to damages from chilling than mature oocytes since they do not contain a metaphase spindle, but few pregnancies from frozen-thawed immature oocytes have been reported [101,102]. IVM, has not been studied extensively in humans and may also have deleterious effects on spindle development and alignment of chromosomes.

- Cryopreservation of ovarian tissue

The idea of cryopreserving ovarian tissue is based on the finding that the ovarian primordial follicles are more resistant to cryo-injury than mature oocytes, because oocytes exhibit a relatively inactive metabolism and also lack a metaphase spindle, zona pellucida, and cortical

granules [103]. Cryopreserving the entire ovary with its vascular supply might help decrease the degree of follicle loss during the initial ischemia period, but at present there is no efficient technique for such a purpose. Although ovarian tissue cryopreservation is not a widely used method, it may be the only acceptable method for any prepubertal or premenarchal female patient receiving chemotherapy or pelvic radiotherapy. The most challenging part of this procedure, is the heterotopic or orthotopic reimplantation of the frozen tissue, a method that is still under investigation.

The first transplantation of cryopreserved ovarian tissue was reported in 2000 [104]. Until today very few pregnancies have been reported using this technique. The risks of ovarian tissue cryopreservation include reimplantation of the primary tumor, malignant transformation, as well as risks related to the invasiveness of the procedure [105]. Limiting factors of this method are its current experimental status, the availability of the procedure in some selected centers and the limited life of the ovarian grafts.

- IVF in women after cancer treatment

At present, the vast majority of patients who underwent chemotherapy or radiotherapy resort to in vitro fertilization (IVF), in order to conceive. Especially for the patients who offered a cryopreservation technique, IVF seems to be the only choice.

Modern assisted reproductive technology (ART) methods, allow the transfer of cryopreserved embryos in a woman's uterine safely, increasing the method's success rates. The introduction of intracytoplasmic sperm injection (ICSI), is of great importance when cryopreserved mature oocytes are used. Exposure to cryoprotecting agents causes hardening in zona pellucida of the oocytes, so that fertilization has to be carried out about three to five hours after thawing while the oocyte remains fertile. ICSI is used for such a direct fertilization. The overall birth rate per cryopreserved oocyte is about 2% when using IVF techniques [106]. Several stimulation agents are used in IVF. Even women with estrogen–sensitive cancer are not excluded from the IVF techniques, as new stimulating agents are used such as tamoxifen or aromatase inhibitors.

Finally, a reference to anti-Müllerian hormone (AMH) in women after cytotoxic therapy is necessary. AMH is used as a marker for the follicles'deposits in women. Several reports to the literature indicate that AMH levels decline with age, predict time of menopause, predict pregnancy after IVF, and are associated with fecundity in the general population. It is of utmost interest that mid-reproductive age cancer survivors who received highly gonadotoxic therapy had AMH levels similar to those in women 40-42 years of age [104], thus providing this group of women with the opportunity to conceive after IVF.

Conclusion

The gonadotoxic effect of chemotherapy and radiotherapy agents is possibly due to mechanisms that still necessitate to be elucidated in the future. It is obvious that the POF rates that have been reported so far differ enormously and are affected by the therapeutic agent(s) used and the patients' age range. Despite the effectiveness of therapy, the methods that are reviewed in this paper for preserving ovarian reserves do not guarantee a 100% success rate in achieving fertility for survivors. It is more likely that different methods need to be applied in combination in order to preserve ovarian reserves and achieve higher fertility rates. This notion is further enhanced by the fact that even less invasive and less expensive methods, such as the use of GnRH in preserving ovarian reserves, still need to be investigated as research results are contradictory. As research is expanding in these areas the more likely is that we will achieve such targets in the future.

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