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Ovarian tissue cryopreservation and transplantation in menopause: new perspective of therapy in postmenopausal women and the importance of ethical and legal frameworks

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Abstract. – Menopausal transition entails a progressive decrease in hormone production by the ovaries that lead to important physical and psychological changes that could significantly affect quality of life. Hormone replacement therapy (HRT) administered from the onset of menopausal symptoms usually improves quality of life and life expectancy. Nevertheless, it is not risk-free. Ovarian tissue cryopreservation (OTC) has been investigated as a potential new strategy for delaying menopause and/or to avoid HRT. This review analyzes the critical points of HRT to assess whether OTC and subsequent reimplantation can affect postmenopausal management.

We assessed available randomized clinical trials in PubMed, Cochrane Library, ISI web of science, and Scopus from August 2021 to November 2022, including studies and trials evaluating the efficacy of OTC in both cancer and menopausal patients, the efficacy of freezing techniques and the possible clinical scenarios that OTC can open, even from the standpoint of legal and ethical issues arising as such innovative techniques become mainstream. Lower duration of the graft and efficacy on estrogen secretions at a physiological and safer concentration of estrogen than conventional HRT based on hormonal supplements. OTC can reportedly trigger estrogen secretions at a lower and safer physiological concentration than conventional HRT. OTC and subsequent reimplantation remain a valid fertility-sparing approach in patients undergoing gonadotoxic treatments. Further studies are needed to better evaluate its safety and efficacy within postmenopausal therapy management and in order to lay out widely shared and evidence-based guidelines and best practices and perform such novel and innovative techniques in a legally and ethically safe fashion, in the best interest of patients and healthcare professionals.

Key Words:
Hormone replacement therapy (HRT), Ovarian tissue cryopreservation (OTC), Menopause, Transplantation, Osteoporosis, Counseling, Guidelines, Medicolegal viability.

Introduction

Life expectancy has been growing considerably over the decades. Women's fertile years however have remained the same, hence women live more years than ever before in a condition of menopause[1].

Entering menopause, estradiol progressively decreases and a consequent increase in gonadotropins occurs, since the negative feedback of ovarian steroids is eliminated[2]. The main source of estrogen becomes the peripheral conversion of adrenal androgens, mainly estrone, which convert into the most important estrogen in menopause.

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Table I. Main indications for HRT.

Main indication for HRT
Patients with symptomatic menopause who do not find relief with symptomatic treatment
Women at risk of osteoporosis or at risk of being diagnosed with idiopathic osteoporosis
Women with premature menopause (iatrogenic or not)

Hormonal changes consequently lead to important physical and psychological changes that could pose serious challenges for women in their later years[3]. The autonomic symptoms, such as hot flashes, insomnia and palpitations are the most common manifestations, in addition to emotional lability, nervousness, reduced libido, difficulty concentrating, memory loss[4], atrophy of the urogenital mucous membranes and dyspareunia[5].

Although these symptoms can be extremely annoying and disabling for the woman, more serious symptoms are often reported, such as a cardiovascular risk. In menopause, cholesterol, LDL and triglycerides increase and HDL decreases, resulting in higher frequency of atherosclerosis, heart attacks and ischemic strokes[6], osteoporosis (the most frequent location of osteoporotic fracture in menopause is the spine, with potentially disabling repercussions)[7] and the risk of cancer (breast cancer primarily)[8].

The use of hormone replacement therapy (HRT) from the onset of menopausal symptoms usually contributes to a better quality of life and even longer life expectancy[9].

Based on the successful use of ovarian tissue cryopreservation (OTC) and its subsequent re-implantation as a fertility preservation treatment in young cancer patient[10,11], the possibility of using the same technique was suggested, programming it well in advance, also to delay the onset of menopause and/or to avoid HRT as it is not without risks[12].

Although such a concept may seem revolutionary when applied to menopause, many questions about its safety and efficacy still linger, and more clinical trials are needed before such techniques can be introduced into the patient's clinical management routine.

The aim of this review is to analyze the critical points of HRT and to assess whether the OTC and subsequent reimplantation can really play a role in postponing menopause and be used as a natural HRT.

Hormone Replacement Therapy (HRT)

Even if the extreme variability of symptoms among women may call for a personalization or, in certain contexts of comorbidities, even question whether to start a treatment, recent findings[13] seem to agree that HRT is the treatment of choice for most women in menopause.

HRT can in fact treat most of the disorders that characterize the onset of menopause and even effectively prevent the medium and long-term consequences of cessation of ovarian activity[14], such as osteoporosis[15].

The onset of HRT must take into account the symptoms, risk factors and individual needs of each patient.

The main indications[16] for HRT are indicated in Table I. The absolute and relative contraindications to HRT are indicated in Table II.

Before starting an HRT, it is essential to carry out a basal mammogram, a cytological examination of the vagina, a transvaginal ultrasound and the evaluation of the lipid and carbohydrate profile. Women who undertake an HRT must have at least one pap-test per year, a clinical and biochemical check-up per year and a mammogram with breast examination at least every two years[17,18].

The main hormones used in HRT are estrogen and progestin, although there are other drugs such

Table II. Absolute and relative contraindication for HRT.

Absolute contraindications for HRT	Relative contraindications for HRT
Hormone-dependent gynecological carcinomas	Hypertriglyceridemia
Positive history of cardiovascular disorders	Dementia
Previous or active venous thromboembolism	Previous cholestatic jaundice
Diabetic vasculopathy	Heart failure
Severe liver disease or liver tumors	Kidney failure
Hypersensitivity to estrogen-progestogens	Previous endometriosis
	Liver hemangioma
	Hyperthyroidism
	Severe hypocalcemia

Table III. HRT pros and cons.

HRT pros	HRT cons
Prevention of osteoporosis and bone fractures disease and ischemic stroke	Increased risk of deep vein thrombosis, ischemic heart
Resolution of vasomotor and neurovegetative disorders	Increased risk of breast cancer after 5 years of therapy
Colorectal cancer reduction	Increased risk of endometrial cancer if estrogen-only HRT is used
The combination of estrogen and progesterone reduces the risk of endometrial cancer	Many absolute and relative contraindications
Is a cheap therapy	
Customizable based on the patient's needs	
Possible different routes of administration	

as androgens, selective estrogen receptor modulators (SERMs)[19] and, only for the treatment of menopausal-induced osteoporosis bisphosphonates, calcium, vitamin D and parathyroid hormone[20].

Natural or equine estrogens are the drugs of choice. They have fewer side effects than synthetic estrogen, which increases the risk of thrombosis and high blood pressure. The route of administration is usually orally, but transdermal administration is strongly recommended for patients with hypertension, liver disease or cardiovascular disorders[21].

The benefits of estrogen therapy are represented by the improvement or disappearance of vasomotor manifestations, disorders due to the atrophy of the genitourinary mucosa (dyspareunia, pollakiuria, urinary incontinence and propensity for vaginitis and cystitis)[22,23] and by balancing neurovegetative and psychomotor disorders[24]. Moreover, considerable benefits arise from the indisputable value of estrogen therapy in the prevention of osteoporosis. The osteoporosis-countering effect is maintained only if estrogen is administered continuously: once the administration is interrupted, the woman begins to rapidly lose bone density, and 5 years after interruption the risk of fracture is comparable to any woman in menopause who has ever taken estrogen[25].

In cases where HRT with estrogen is absolutely contraindicated or not tolerated by the patient, other solutions can be considered. Progestin therapy is the most used in this area, as in addition to being effective against vasomotor disorders and some manifestations that undermine the psychological balance of women, they are also protective against endometrial cancer and mastopathy of a benign nature. Furthermore, although with a different mechanism than estrogens, progestogens, even taken alone, are effective in preventing osteoporosis, facilitating new bone formation rather than decreasing its resorption (an effect brought about by estrogens). When taken in combination with estrogen, the effect on osteoporosis is mutually amplified.

The main adverse effects of progestogens are related to their impact on lipid metabolism, since they increase LDL and decrease HDL, to the higher thromboembolic risk and to possible painful symptoms in the breast. They can also cause significant mood disturbances and uterine bleeding.

However, the possibility of using non-oral routes of administration, for example transdermal, limits the side effects[13].

Although conventional HRT is used to counter the various adverse effects of menopause, its use is associated with certain controversies regarding an increased risk of breast[26], endometrial[27], serous and endometrioid ovarian cancer[28], venous thromboembolism, heart attack and ischemic stroke[29,30].

Fertility Preservation: Younger Better than Older

Knowledge in the field of biotechnology is constantly evolving, particularly in fertility cryopreservation, even more so in light of the most recent achievement: ovarian tissue cryopreservation for

patients who undergo fertility preservation procedures, following a diagnosis of genital sphere carcinomas at a young age[31], which entails significant psychological repercussions[32] and usually needs fertility sparing surgery. Counseling is therefore of utmost importance, in light of the possible life-changing consequences such measures could produce[33-36] in addition to the legal and ethical viability of each intervention, which will be further discussed later on. As for freezing techniques, vitrification has been shown to be more effective than slow cooling, as it is a procedure that prevents ice crystals formation within cells, leaving cell domains intact[37,38]. More and more women today resort to egg donation programs due to their advanced age. Egg donation has always produced the highest pregnancy rates among all assisted reproduction methods, mostly due to the selection of oocytes from healthy young donors.

In particular cases of young donors with polycystic ovarian syndrome (PCOS) that usually have ovarian hyper response to gonadotropin stimulation, a GnRH antagonist is recommended to avoid ovarian hyperstimulation syndrome (OHSS). It should be administered twice the day before hCG trigger combined with a step-down protocol[39] and supplementation with inositol before and during the stimulation[40]. Sometimes, embryo-transfer timing, specific techniques and vitamin D can improve fertilization and pregnancy rate[41,42].

Scientific findings[43] show how supplementation with inositol contributes to ovulation recovery in patients with ovulation defects. Furthermore, inositol supplementation has been demonstrated to be helpful in pregnancy from PCOS patients to manage gestational diabetes[44] and in the menopausal transition[45].

Outlining an effective therapeutic pathway for infertile women is certainly very challenging and should not be underestimated. Taking a "gender-based approach"[46] is indeed instrumental in ensuring a sound decision-making process, as is taking into account new, innovative techniques aimed at assessing prognostic and diagnostic factors based on molecular biology and more reliable clinical and prognostic classification of underlying disease such as cancer[47-49].

Ovarian Tissue Cryopreservation (OTC)

Based on the recent encouraging results of studies[50-54] conducted on the OTC, particularly several involving patients with oncological or autoimmune conditions predisposing them to premature ovarian insufficiency and/or infertility[50-52], the OTC and its subsequent transplantation could be proposed as an alternative to HRT[53]. This allows to avoid all the side effects related to the latter, as well as a possible treatment to delay menopause, provided that all precautions aimed at patient safety are prioritized at all times, as in any other major organ transplant[54].

One way of standardizing the technique is to identify the correct age at which the removal of the ovarian tissue should be performed, in order to have an appropriate number of oocytes needed to restore ovarian function. Wallace and Kelsey[55] have shown that by the age of 30, only 12% of the pre-natal oocyte population is still present, the figure is further lowered to 3% if the sample is taken at the age of 40. It is therefore clear that the earlier the sample is taken, the greater the quantity of oocytes is[56]. According to Donnez and Dolmans[9] the best age would be 25 years. Relying on a large number of oocytes in the collected tissue is also essential to deal with problems related to the technique itself. The technologies currently in our possession for the thawing and revascularization process are in fact not suitable for preserving 100% of the collected oocytes[57-59].

The technique is an elective surgery, which requires the ovarian tissue to be removed, cryopreserved, and re-implanted at the onset of menopause. Clearly this is an invasive therapeutic procedure which involves at least two interventions: one of sampling, conducted with a laparoscopic procedure, and one of re-implantation[59].

Reimplantation can take place in the orthotopic area if the ultimate objective is to restore the patient's fertility as well, otherwise, for ease of access, a heterotopic reimplantation in the muscles of the forearm, abdominal wall or breast muscles would be preferable[60-62].

In addition to requiring an extremely less invasive surgery compared to an abdominal-pelvic laparoscopy, the implant in the heterotopic site allows the repetition of more reimplants, prevents pregnancy in elderly patients and above all, allows to perform such technique even in a patient with severe abdominal-pelvic adhesion syndrome. However, the heterotopic implant has some disadvantages[63]: patients are limited in their movements due to the need to reduce excessive pressure at the level of the reimplanted ovarian tissue, the growth space of the follicle can be smaller and therefore be hindered, the vascularization can be variable site to site, and the tem-

perature could affect the correct function of the graft. Therefore, there is no election site to date. All this exposes the patient to a double surgical risk, albeit extremely limited. Leaving out the well-known complications of a laparoscopy on a healthy patient, the complications associated with reimplantation occur rarely, around 2 patients out of 100[64,65], and these are mainly represented by cyclic pain at the time of ovulation, swelling and discomfort at the time of follicular growth if the site of choice is the heterotopic one[66].

The duration of the implant both in the orthotopic and heterotopic site can last up to 7 years[67,68], although this strictly depends on the age of the patient and on her ovarian reserve at the time of collection, on pre-existing conditions and any treatments that can reduce ovarian function, such as chemotherapy[69]. Thanks to the improvement of the sampling technique, the accurate selection of patients and effective thawing of the cryopreserved tissue, the duration of the graft is substantially longer than in the past. Ovarian grafts in fact used to be estimated to restore ovarian function only for 2-6 months[70]. However, further studies are necessary to establish the most correct method for thawing and revascularization of the cryopreserved ovarian tissue, in order to limit the loss of oocytes as much as possible, and further extend the period of operation of the graft[70-72].

Although at present there is no indication to use this method to reduce symptoms associated with menopause or to prevent menopausal osteoporosis[73], Kristensen and Andersen[74] have suggested that transplantation of previously cryopreserved ovarian tissue, under the stimulus of the hypothalamus-pituitary-ovarian graft axis, may lead to estrogen secretions at a physiological, lower and safer concentration of estrogen than conventional HRT.

Literature has shown the effectiveness in restoring ovarian function after transplantation of cryopreserved ovarian tissue. However, for some patients who undergo such a clinical intervention, even if the adverse effects of conventional HRT are fewer, the resolution of menopausal symptoms does not occur quickly[75-77]. In any case, preliminary findings[78] on the prevention of menopausal disorders, osteoporosis and its consequent fractures, and the reduction of cardiovascular risk, are remarkably promising.

Although we are encouraged to undertake new clinical trials that can establish the real efficacy of OTC as an HRT instead of conventional HRT, many doubts are still unresolved. The risk of breast cancer, certain to be increased by conventional HRT, could also be raised by OTC, as it would still restore ovarian activity beyond its physiological duration. It is also necessary to investigate the possibility of cancerization of the reimplanted ovarian tissue. Other unresolved dilemmas are the actual need for this technique to be applied to hysterectomized patients, since still receiving progesterone for a long time could be dangerous from a cardio-vascular standpoint.

However, it should be emphasized that naturally produced progesterone has a decidedly lower impact than that taken externally on the pathogenesis of breast cancer[15]. The technique could instead be extremely useful for non-hysterectomized patients, since estrogen associated with progesterone is protective for endometrial cancer, unlike estrogen alone which is a risk factor[10,27]. Finally, there remains the important question of evaluating the risk-benefit ratio. For a young cancer patient the benefits can be certainly higher than the costs, both in terms of preserving fertility and of the very short time that the cryopreserved ovarian tissue requires to stay, but for the woman who wants to delay her menopause, or wants to take this therapeutic route rather than the classic HRT, the risks could exceed the benefits, since many aspects of the technique have not yet been clarified.

The Importance of Clearly Defined Evidence-Based Standards for Innovative Techniques

Considering how high the stakes are for patients whose residual chances to achieve parenthood, (especially when such chances may depend on novel, highly innovative techniques such as OTC and transplantation) it is of utmost importance to be able to rely on clean-cut, widely shared and agreed standards, in the form of guidelines and best practices[75]. Research-based criteria delineating patient selection, contraindications and risk-factors are valuable in terms of providing a degree of objectivity for doctors and patients alike and guaranteeing the legal and ethical sustainability of each prognostic and therapeutic intervention. Hence, adherence to solid standards of care (which must be documented, i.e., provable, at all times) is essential in order to provide doctors and healthcare facilities with evidentiary elements that can shield them from negligence-based malpractice allegations and lawsuits[79]. Obstetrics and gynecology, including ART procedures and pregnancy/childbirth, are

Table IV. Cryopreservation of ovarian tissue pros and cons.

OTC pros	OTC cons
Replaced ovarian tissue could re-establish pulsatile hormone secretion	It includes at least 2 surgeries
Hormone levels could be physiological, therefore safer than traditional HRT	The duration of the ovarian graft is difficult to predict
Naturally produced hormones have a lower risk profile than synthetic hormones	Current knowledge on thawing and revascularization technique does not allow 100% conservation of the collected oocytes
Fewer side effects than traditional HRT while maintaining the same benefits	The costs are higher than HRT
Possibility of late pregnancy with or without IVF depending on the reimplantation site	Its effective application to manage the symptoms of menopause has not yet been established, in terms of risk or efficacy

in fact among the medical specialties most at risk for litigation[80,81].

In light of such dynamics, the relevance and role of reproductive counselling in the management of the cancer patient, i.e., an essentially multidisciplinary effort, cannot be overlooked, as stressed by recommendations and guidelines by several medical associations[82-84]. Against such a backdrop, the medical intervention is defined and validated by a legal-ethical context of rights, obligations, and responsibilities to which doctors and all healthcare operators are required to adhere, fully and universally legitimized by informed consent, in the absence of which healthcare personnel are not authorized to perform any clinical intervention[85]. Reproductive counselling therefore rises to the role of an essential information because it is an essential part of the counselling stage, at which point doctors have a responsibility to make sure that all potentially viable therapeutic avenues are known and understood by their patients[86,87]. That is even more essential in light of the fact that some procedures such as OTC are not yet well-established in clinical practice and can have a bearing on the fundamental human right to reproductive health and freedom. Furthermore, when experimental interventions are involved, patient choice is likely to be complicated by unsolved doubts and uncertainties[88]. That makes a well-balanced and thorough approach to the counseling process even more consequential and meaningful in terms of ensuring that the patient's self-determination, freedom of choice and exercise of reproductive rights are best served[89].

Conclusions

Conventional HRT is currently the most effective avenue in the treatment of menopausal disorders – both autonomic and atrophic – and in reducing the risk of osteoporotic fractures and colorectal cancer. However, prolonged use of this type of therapy is associated with various side effects, the most important of which is the increased risk of breast cancer. In addition, in the first 5 years of therapy, thromboembolic accidents constitute the most serious risk. For non-hysterectomized women, estrogen-only therapy is not recommended due to the increased risk of endometrial cancer. It would therefore be preferable to associate progestin, by virtue of its protective effect on the development of this tumor. However, although most of these data come from studies in patients taking HRT orally, alternative routes of administration could significantly lower the risks (Table III).

OTC and its subsequent reimplantation remains a valid approach to preserve fertility in cancer patients or patients undergoing gonadotoxic treatments. Since the role of OTC is increasingly establishing itself as a fertility-sparing approach, as far as menopause is concerned, re-establishing a natural pulsatile hormonal secretion, could soon constitute a replacement for traditional HRT for the management of menopausal symptoms and for the reduction of HRT adverse effects (Table IV).

Finally, it is essential to lay out a more thorough and evidence-based evaluation of OTC safety and efficacy. Many clinical settings are still required

to consider OTC as a treatment of choice in the management of postmenopausal therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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