REVIEW ARTICLE

Ovarian Tissue Microfragmentation and Exposure to Autologous Growth Factors *In Vitro* and Reproductive Outcome after Orthotopic Retransplantation

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Abstract

Generally speaking, it is estimated that infertility affects about 186 million people worldwide. Infertility is still considered to be a female social burden, even though male fertility can be regarded as a half, or even more, of all cases of global infertility.

This review aims to analyze the literature regarding the influence of growth factors on ovarian tissue cells after *in vitro* exposure and the establishment of normal reproductive and endocrine function after retransplantation.

Primary ovarian insufficiency (POI) is one of the possible causes of infertility in women, affecting about 1% of the general population. Some of the specifics of premature ovarian failure (POF) are amenorrhea, elevated gonadotropin levels in women under 40, and also hypoestrogenism. Procedures such as ovarian transplantations can in fact lead to fertility restoring and endocrine function restoring. Ovarian tissue transplantation can be put into two categories, based on the tissue type that is planned to be transplanted: as ovarian cortex transplantation or ovarian transplantation.

The process of freezing-thawing ovarian tissue is well known and researched, but the usage of cryopreserved ovarian cortex in fertility restoration still remains a significant challenge. Total effects of growth factors must be explored to a larger extent, but it is certainly encouraging that the known results are, by this day, quite satisfying. The beneficial effect on later follicle development or fertility restoration has yet to be researched and fully established.

Keywords: Growth factors, Orthotopic ovarian transplantation, Primary ovarian insufficiency, Sterility.

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Introduction

As a term, sterility is considered to be a certain state of inability to conceive, after multiple years of unprotected intercourse. The most common examples related to infertility are ovulation issues, difficulties and challenges with the fallopian tubes, endometriosis, problems with uterine etiology, and, not so rarely—hormonal difficulties, as well as disorders of spermatogenesis and azoospermia. Ovaries are dynamic organs which show large structural changes in a short period during each reproductive cycle.[1,2]

Sterility is by far one of the most difficult health problems in various communities. The prevalence of this problem has significantly increased its importance.[3] In the second decade of the new millennium, infertility has been an important and globally spread problem. It is estimated that infertility affects 8–12% of couples in reproductive age, all over the world, with about, currently reported, 9% of global average. In the United States of America, about 6% of women in their reproductive age, are facing the problem of infertility. Depending on several different factors, infertility rates tend to be higher in certain regions, reaching up to 30% in some populations. Regions with high infertility rates are, for example, South Asia, sub-Saharan Africa, North Africa, Central and Eastern Europe, Central Asia, and the Middle East.[4] In total, it is estimated that infertility is affecting approximately 186 million people

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worldwide. Unfortunately, infertility is considered to be a female social burden, although male infertility contributes to more than half of all cases of global infertility.[4]

This review paper aims to analyze the literature on the influence of growth factors on ovarian tissue cells after *in vitro* exposure and the establishment of normal reproductive and endocrine function after retransplantation.

Literature Review

One of the possible causes of infertility is POI, which is affecting 1% of the human population. The specifics of POI are amenorrhea, hypoestrogenism, and elevated gonadotropin levels in women under 40. Thus, a significant number of women in their 40s are facing the problem of reproductive ability, losing reproductive ability, and different symptoms which affect overall health and, furthermore, quality of life. Ovulatory dysfunctions make up about 30% of infertility in females. Fortunately, ovarian transplantations can restore both fertility and endocrine function. Ovarian transplantation methods can be put into two categories: ovarian cortex transplantation and ovarian transplantation. Considering the transplantation, ovarian transplantation can be categorized as orthotopic or heterotopic. When it comes to the sources of transplant therapies, transplantation procedures can be divided into autologous, allogenic, and xenotransplantation.[5–7]

Cryopreservation of ovarian tissue is a high-potential experimental technology, that has several advantages. It allows the storage of a large number of primordial and primary follicles, can be quickly performed at any time of the menstrual cycle, and also it is the only option available to preserve fertility in children. The procedure has been proposed as an option for fertility preservation for various indications in a growing number of centers around the world.[8–10] The first orthotopic transplantation of cryopreserved ovarian tissue, and regarding autotransplantation, was reported by Oktay.[11] Since then, various sites have been studied in humans, in order to restore both ovarian function and fertility. Orthotopic sites referred to transplantation of ovarian tissue, in the ovarian peritoneum and/or in the remaining ovary. Because of its negligibly invasive surgical aspect and accessibility (forearm or abdominal wall) it is mostly chosen as a heterotropic site, sometimes associated to transplantation in an orthotopic site.[12,13] Different heterotopic sites have been researched, and they include uterus, abdominal muscle, superficial fascia, chest muscles, and also the space in between breast tissue.[14] It is also worth mentioning subperitoneal tissue below the abdominal fascia, between the pubic bone and navel, as one of the researched heterotopic sites.[15] Even though mentioned heterotopic sites have been shown to have a certain effect on ovarian function restoring, there have not been reported clinical pregnancies from collected oocytes. Heterotopic sites are proved to be effective in restoring ovarian function, but no clinical pregnancy has been reported from collected oocytes, even though embryos were obtained and transferred. In all cases of birth, after the transplantation of ovarian tissue, fertilized eggs originate from tissue transplanted at an orthotopic site: peritoneum in the ovary[16] or the remaining ovary.[17]

Poor gonadotropin stimulation responses can be encountered in approximately 9–24% of women that are subjected to *in vitro* fertilization (IVF). Double ovarian stimulation is able to produce more oocytes/embryos in one stimulation cycle in a short time period, which makes it a highly significant and potent strategy for women that are facing low ovarian responses. Generally speaking, there are two follicle types: preantral (primordial, primary, secondary and tertiary); as well as the antral (graafian, small, medium, large, preovulatory) follicles. The preantral stage is gonadotropin independent phase, while the antral phase is gonadotropin dependent stage. In the preantral stage, follicle development is influenced by intraovarian growth factors through two equally important mechanisms: autocrine/paracrine regulation. Growth factors within the ovary include nerve growth factors, KIT ligands and their receptors, members of the transforming growth factor-β superfamily (TGF-β), growth differentiation factor 9, bone morphogenetic protein 6 (BMP6), BMP4, BMP7, and BMP15, and fibroblast growth factors (FGF). Above mentioned growth factors may have a meaningful effect on local regulation and modulation of follicular development and, of course, follicular selection.[18]

A number of growth factors, which include FGF, TGF-β-a, or vascular endothelial growth factor (VEGF) are mostly involved in the process of blood vessels invading tissue. It has been proven that FGF and VEGF can cultivate development of follicles, both for *in vivo* and *in vitro* setup. Regarding *in vitro* conditions, VEGF stimulated preantral follicle growth in cattle and rat models. Fibroblast growth factor had a meaningful effect on promotion of early follicle growth in mice, goats, and most importantly, on humans.

The manner by which VEGF and FGF promote follicle development is regarded to regulating the cytokinetic interaction. In an *in vivo* setup and model, direct injection of VEGF into the ovary of mice promotes follicular development and affects reducing apoptosis. Local inhibition of VEGF activity increases ovarian apoptosis, which results in more follicles that lead to atresia. Fibroblast growth factor is somewhat considered to be crucial for follicle activation and development, while the FGF administration may promote the development of human early ovarian follicles during *in vitro* growth. Fibroblast growth factor improves VGF expression of VEGF in endothelial cells and certain cell types. Regarding an *in vitro* angiogenesis model, both FGF and VEGF have presented certain angiogenic effects on human microvascular endothelial cells in a dose-dependent manner, and when combined, the two factors acted synergistically.[19] Erythropoietin may improve the survival rate of the tissue that has been transplanted, as it promotes the differentiation and proliferation of erythroid progenitor cells and it also prevents apoptosis.[20]

Both hormonal and molecular analyses have been directed to discover the mechanisms underlying structural changes in the ovaries. Unfortunately, the specific mechanisms are yet to be determined. Discoveries and findings in mechanobiology have led to better understanding of the contribution of physical forces and changes in the mechanical properties of cells and tissues to physiology and pathophysiology. The key mechanism in mechanotransduction is Hippo signaling pathway, and it provides an explanation of the molecular mechanisms in which cells respond to mechanical signals that regulate cell proliferation and apoptosis in order to sustain organ sizes in an optimal order and manner. The exact way of Hippo signaling pathway influencing ovarian development regulation has been demonstrated in the recent period. Because the ovarian cortex fragmented into small parts/cubes, it affected the cytoskeletal actin dynamics and it actually altered it in a certain manner, which further led to CCN growth factor production, as well as the production of antiapoptotic BIRC. These mentioned factors increase the growth of secondary follicles in both *in vitro* and *in vivo* setups.[1]

When it comes to releasing growth factors, platelets have a huge role in that: platelet-rich plasma (PRP) is a potent concentration of autologous human platelets (3–5 times higher than baseline plasma levels) that consists of hormones, cytokines, adhesion molecules, coagulation factors, chemokines, integrins, and growth factors, such as platelet-derived growth factors (PDGF-AA, PDGF-BB, and PDGF-AB), TGF-β1 and TGF-β2, insulin-like growth factor 1, VEGF, epithelial growth factor and epidermal growth factor.[18]

Above mentioned growth factors affect cell proliferation by stimulating it, and besides that, they are considered to be an extremely important factor in blood vessel forming, collagen synthesis, and extracellular matrix. These growth factors stimulate cell proliferation and play a significant role in the formation of blood vessels, collagen synthesis, and extracellular matrix formation. PRP has a significant role in promoting cell division, proliferation, differentiation and migration, angiogenesis, extracellular matrix remodeling, tissue regeneration and healing. So far, there are new perspectives presented regarding ovarian rejuvenation attributable to the presence of ovarian stem cells and germline in the ovarian surface epithelium having the ability to differentiate oocytes under certain conditions, as well as the existence of endothelial and vascular growth factors in PRP. PRP may be used to create primary ovarian follicles and consequently antral follicles in old age, especially in women with the poor ovarian response, premature ovarian insufficiency, and ovarian dysfunction caused by chemotherapy before and after menopause.[18]

Due to different environmental, genetic, and other factors, there is a significantly increased incidence of malignant diseases affecting people of all age groups and, today, it is estimated that 2% of the female population under the age of 40 will suffer from some type of malignant disease.[19] Thanks to medicine and drug development, modern therapies for malignant diseases treating have enabled people to have a higher survival rate and extended life expectancy. However, the treatment of malignant diseases leaves behind a large number of endocrine disorders, including infertility.[21,22] Studies show that one in six women will have problems conceiving after oncology therapy.[23] The risk of impaired fertility differs from one patient to another, increasing with the patient's age, and relating to the dosage, type, duration of chemotherapy and radiotherapy. Thus, preservation of fertility has become a high priority for patients who must be underlaid to an aggressive treatment for certain malignant diseases.[24,25] The likelihood of miscarriage after oncotherapy in women over 25 years of age is estimated to be only 5%.[26] In order to preserve fertility chances, while planning gonadotropic treatment, cryopreservation of ovarian tissue and reimplantation into the pelvic cavity are performed as the chosen methods. According to the available studies, the pregnancy rate after autotransplantation of cryopreserved ovarian tissue to orthotopic sites is about 30%.[25–29]

At the time, autologous ovarian tissue transplantation is mostly used in patients with cancer. Prior to performing chemotherapy and radiotherapy, normally functioning ovarian tissue is preserved and by doing that, a patient can be sure that ovarian tissue will not get affected by mentioned therapies. In sequel to therapy, ovarian tissue is returned to the patient's body in order to restore the endocrine and reproductive functions of the ovaries. According to data from five centers that provide autologous transplantation, the percentage of patients that conceived after the transplantation technique was approximately 29%. It is widely recommended for this technology to be used in cancer centers to preserve fertility, suggested mostly for preadolescent girls or in cases in which it is not recommended for chemotherapy to be postponed, due to the course of illness and its aggressiveness.[5]

Cryopreservation should not only be intended for women who are facing malignant diseases, but also for cases where hematopoietic stem cell transplantation is indicated for nonmalignancies.[30–32] Benign diseases, such as recurrence of ovarian endometriosis or recurrent ovarian mucinous cysts, can also be considered as a matching indication for cryopreservation methods. Drastic types of endometriosis are known to be correlated with decreased oocyte quality[33] and lower serum anti-Müllerian hormone concentrations.[34] Women facing different sorts of autoimmune diseases such as lupus nephritis, genetic disorders, or benign ovarian diseases that require oophorectomy, may also be suitable candidates for fertility preservation. Women with no malignancies, but facing some kind of infertility difficulty, represent closely 20% of the indications in world's population of people who require undergoing methods of fertility preservation.[35,36] Cryopreservation of ovarian tissue shortly before oncological treatment, may be considered a promising method because a large number of follicles can survive the freezing and thawing processes.[37] Donnez

et al. reported the first live birth after autotransplantation of human ovarian tissue in 2004.[16] The first successful live birth after retransplantation of cryopreserved ovarian tissue, in Germany, was reported in 2012.[38]

Primordial follicles stay dormant in the ovaries and quite a few intraovarian factors have the potency to modify those resting states. Even though the precise mechanisms, that activate a certain number of dormant follicles, are yet to be discovered, studies show that intracellular signaling mechanisms are major for activation of dormant primordial follicles.[39]

Premature ovarian failure is a heterogenic disorder which is characterized by stopped function of the ovaries, which leaves the ovaries without a chance to produce typical and standard amounts of estrogen, which leads to inability to release eggs in the normal manner. This disorder happens to women under the age of 40. The number of residual follicles decreases below the threshold (less than 1000), and normal activation of follicles does not occur, which then leads to anovulation and amenorrhea. POF that affects 1% of women can be caused by autoimmune ovarian damage or due to genetic aberrations which mostly include the X chromosome, autosomes, or specific genes. Factors that include ovarian surgery, radiation, and chemotherapy interventions, may also cause POI. POF patients manifest postponed spontaneous ovulation at an early age, so the habitual infertility treatments do not lead to wanted results. Cancer patients are indeed exposed to chemotherapy or radiotherapy that can be harmful to germ cells; cryopreservation of ovarian tissue, mature oocytes, and embryos are available options that may be potentially considered. Some of the most effective treatments for infertility in patients with POF include IVF and embryo transfer with the use of donor eggs. Although there may be noticeable amounts of residual follicles in ovaries in POF patients, it is challenging for those follicles to grow spontaneously. We are satisfied and honored to say that we recently developed a new infertility treatment which is called *in vitro* activation (IVA), and it allows POF patients to conceive their eggs with the help of artificial activation of residual dormant follicles. In this review, we summarize the potential use of IVA as a new treatment for infertility in patients with POF.[39]

Ovarian resection and microfragmentation in patients that struggle with the problem of infertility, or polycystic ovary syndrome, indeed managed to induce follicle growth. In women that are undergoing sterilization, the ovarian cortex is found to be fragmented, so it can permit better freezing and transplantation, aiming to preserve fertility. The subsequent transplantation of fragmented cortexes is narrowly related to follicle growth. These methods include, for example, the rupture of the ovarian cortex, that can further indicate the induction of follicle growth by changes in mechanical stress. IVA may increase the number of mature eggs for the treatment of infertility, but it does not have any relation or effects on the decrease in oocyte quality. It is interesting that, even though observations regarding POF patients do in fact have implications, there have been noted several, and not so rare, spontaneous recoveries of the menstrual cycle and following pregnancies.[39]

The only option for fertility restoring, by this day, is the usage of cryopreserved ovarian tissue.[38,39] The ovarian cortex transplantation is managed without vascular reanastomosis, it makes the tissue perfusion depend on the new blood vessel invasion.

The period of time that determines achievement of perfusion in the tissue that has been transplanted is actually crucial for the survival of the follicles, as well as the graft endurance. One of the following critically important factors for the neovascularization process and follicle survival is stroma integrity. Ischemia is tolerated by primordial follicles and can occur for minimum 4 hours during tissue transport,[39–42] and the stromal cells surrounding follicles tend to be more subjected to ischemia when compared to primordial follicles.[43]

The procedure of exposition of human ovarian tissue to freezing and thawing process has been studied to a large extent. Follicle survival rates are estimated to be approximately 70–80% right after exposure to slow freezing with suitable cryoprotectants. The slow-freezing cryopreservation method may act on the outcome of reproduction, after transplantation.[44]

Conclusion

The process of freezing-thawing ovarian tissue is now relatively well established and well-studied, the usage of cryopreserved ovarian cortex in fertility restoration is yet presented as a true challenge. The effect of growth factors is yet to be explored to a large extent, but up to this day, the results are encouraging. The impact of growth factors on further follicle development or fertility restoration must still be confirmed through additional research and numerous studies.

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