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REVIEW

# The Artificial Ovary: the Next Step in Fertility Preservation in Cancer Patients

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**ABSTRACT**

*The cryopreservation procedure of ovarian tissue is used for subsequent transplantation to preserve fertility in cancer patients. In the case of cancers with possible ovarian damage, due to the increased risk of transmission of malignant cells in the cryopreserved ovarian tissue, after remission of the disease, the transplant cannot be performed due to the high rate of recurrence. Thus, to resolve fertility preservation in these cancer patients, making an artificial ovary that could be transplanted under maximum safety conditions was necessary. This was not easy to achieve because it was essential to develop a porous and rigid matrix that could encapsulate and protect the ovarian follicles and, at the same time, create an optimal neuroendocrine environment. The present article analyzes the technological progress in creating an artificial ovary, the opportunity for transplantation, the proper counseling of these patients, and the prognosis regarding using this modern technique to preserve fertility.*

**Keywords**: artificial ovary, cancer patients, fertility preservation, transplantation, *in vitro* culture, preantral follicles, biomaterials, fibrin matrix.

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## INTRODUCTION

Cancer treatment, in particular chemotherapy, affects both endocrine and reproductive function by affecting follicular DNA, accelerating ovarian follicles or impairing ovarian vascularization (1).

Experimental studies on the clinical application of artificial reproductive organs (ovary, uterus, gametes) are promising, mainly in animal models, representing a solution for infertility cases, especially in cancer patients (2). Artificial human ovarian technology may be a viable fertility restoration strategy for cancer and premature ovarian failure patients. In recent years, the survival rate and prognosis of young adults diagnosed with cancer have increased through early diagnosis and improved the efficiency of chemoradiotherapy treatments. This trend was followed by the need to diversify the options for maintaining those patients' fertility. Gonadotoxic chemotherapy induces premature ovarian failure, which requires the development of fertility conservation strategies (ovarian transposition, cryopreservation of ovarian tissue, oocytes, and embryos, or transplantation). Unlike the alternatives offered to young adults diagnosed with cancer, in the case of prepubertal girls, only ovarian tissue cryopreservation and transplantation can be used to preserve fertility (3, 4).

In patients with a low risk of ovarian metastases, cryopreserved ovarian tissue transplantation has effectively restored ovarian function, resulting in more than 60 live births to date. Conversely, in patients with a moderate/high risk of ovarian metastasis, the option is an artificial ovary because the risk of recurrence is given by the possibility of reimplantation of malignant cells (Table 1) (5, 6).

Restoring fertility in patients with cancer after remission is a particularly important issue. This is possible in patients without risk of ovarian damage through cryopreservation and ovarian tissue transplantation techniques. In contrast, in cancer patients with ovarian involvement in whom ovarian tissue transplantation cannot be performed due to the increased risk of disease recurrence, the option is to perform an artificial ovary transplant (7, 8).

Thus, the artificial ovary involves the isolation of the follicles after cryopreservation of the ovarian tissue before the gonadotoxic treatment and the creation of an artificial matrix in which these follicles are encapsulated. In addition, supporting the reproductive function of these follicles involves restoring normal endocrine function (6).

In an attempt to construct an artificial ovary from cryopreserved ovarian tissue, Schmidt *et al* concluded that the use of tumor dissociation enzyme (TDE) compared to Liberase TM did not increase apoptosis and follicular necrosis after enzymatic degradation of ovarian tissue (9). According to the study conducted by Chen *et al*, the technique of using TDE may result in the isolation of a larger number of primordial follicles with a smaller diameter and low intensity of oxidative stress compared to Liberase Dispase High (DH) (10).

In recent years, several matrix schemes have been tested on the strategy of developing an artificial ovary, starting from experimental studies with mouse follicles. The best artificial model is based on a mixture of thrombin and fibrinogen to create a rigid architectural composition necessary for encapsulating and grafting secondary follicles. Studies have shown that ovarian stiffness causes functional changes in the follicle (11, 12).

Another study considered the possibility of encapsulating and grafting primary follicles. Thus, the most accurate knowledge of the physiology and anatomy of the ovary is the basis for designing a functional artificial matrix that can successfully replace natural ovarian tissue (13, 14).

Our study aims to show the importance of creating an artificial ovary in cancer patients with possible ovarian damage and to present the role of advising these patients on the possibility of preserving fertility.

**TABLE 1.** Risk of ovarian metastasis according to cancer type (6)

| Low risk | Moderate/High risk |
|---|---|
| • Squamous cell carcinoma of the cervix | • Adenocarcinoma of the cervix |
| • Breast cancer (stage I–II ductal type) | • Breast cancer (stage IV lobular type) |
| • Hodgkin lymphoma | • Non-Hodgkin lymphoma |
| • Wilms' tumor | • Leukemia |
| • Osteogenic carcinoma | • Colon cancer |
| | • Neuroblastoma |
| | • Burkitt lymphoma |

### Transplantable artificial ovary

The initial stage involves an ovarian tissue biopsy before starting gonadotoxic treatment, followed

by cryopreservation of the entire fragment or isolation of the preantral follicles. The latter can be cryopreserved isolated or not, both techniques not affecting their functionality. Isolation of preantral follicles is preferred, as cryopreservation may affect the number and viability of ovarian cells after isolation. The follicle isolation protocol must exclude the existence of malignant cells from the follicular suspension (15, 16).

The artificial ovary is based on an *ex vivo* experimental technology in several stages to produce mature oocytes for *in vitro* fertilization (IVF). Sequential technology takes place in three stages of culture based on fresh or frozen ovarian tissue. Initially, the development of primordial follicles in ovarian cortical tissue cultures is followed by their isolation from the ovarian cortex. Subsequently, after isolation of the ovarian follicles from the cultures, growth occurs in a biodegradable 3D matrix consisting of matrigel, fibrin, or alginate. Next, *in vitro* isolation of oocytes from cultured follicles is performed. The last stage aims to obtain mature oocytes from cultured immature oocytes, to be fertilized or frozen (17) (Figure 1).

This experimental option can benefit several categories of cancer patients undergoing gonadotoxic treatments: alkylating chemotherapy, abdominal/pelvic radiation therapy, or irradiation of the body/skull.

In a study on surgically sterilized mice after a xenograft of a 3D-printed ovary prosthesis, Laronda *et al* demonstrated a normal endocrine and reproductive status (18). Thus, restoring fertility in cancer patients by creating a 3D-printed artificial ovary is a viable option for preserving fertility. To achieve the 3D biopolymeric construction of an artificial ovary to encapsulate the preantral follicles, a porous, rigid and biodegradable structure is needed to develop the follicles neoangiogenesis and the processes of cell migration.

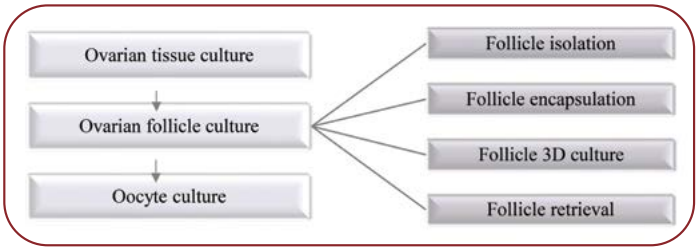


**FIGURE 1.** Algorithm of construction of an artificial ovary

Woodruff highlighted the importance of implementing nanotechnology, inorganic chemistry, and biomaterials in creating and maturing extragonadal follicles with special clinical involvement. The EVATAR system is a microfluidic assembly that interconnects the ovary, the uterine body and the fallopian tubes, which has a duration of operation of 28 days and reproduce the endocrine environment of the reproductive tract (19). The biomaterials used to make an artificial ovary can be natural or synthetic polymers. The natural polymers for ovarian follicle transplantation were the first to be used. They are enzymatically biodegradable, rigid in structure, and have high cell interaction and adhesion (20, 21).

## Ovarian follicle transplantation

### *Natural polymers*

Collagen derived from connective tissue, also found in the ovarian cortex, can be used for a matrix due to its increased resistance and low immunogenicity. Not many studies have been conducted despite promising results (22, 23).

Plasma clots were used as an autologous biomaterial with excellent cell adhesion compatibility, proliferation and differentiation, and growth factors. Studies have shown that plasma clot matrices may show changes in composition and increased biodegradation after transplantation, compromising follicles (24).

Another matrix consists of a fibrin gel made up of fibrinogen and thrombin. This gel has been used as a biopolymeric adhesive for surgery due to its reduced inflammatory process, good degradation, and the promotion of neoangiogenesis. Creating a biopolymeric matrix as active as possible from a functional point of view can be achieved by changing the concentration of fibrinogen and thrombin, respectively. This will cause a change in the rate of degradation, stiffness, and fiber network (25, 26). Thus, Luyckx *et al* made a matrix for the artificial ovary using a fibrin network, obtaining antral follicles after transplantation (27, 28).

Frequently during tissue processing, most encapsulated follicles are located at the edge of the fibrin clot, which is associated with a loss of the number of follicles, which requires rapid grafting of the fibrin clot (29).

The transplant was responsible for the resumption of hormonal cyclicity, objectified by the decrease of FSH and the presence of the cor-

pus luteum. Other studies have added hyaluronic acid to the fibrin network acid to improve the survival of the preantral follicle, platelet-activated extract (30) rich in angiogenic growth factors (VEGF, PDGF) (31, 32).

The use of alginate hydrogel in the encapsulation process of isolated follicles is a procedure applied to transplant an artificial ovary. It is derived from brown algae and bacteria, a polysaccharide based on two monomeric units – β-d-mannuronate and α-1-guluronate (33, 34). Alginate has been widely applied as a matrix or delivery system in regenerative medicine due to its biocompatibility, non-antigenicity, and versatility. Vanacker *et al* used SLM alginate as a biomaterial for transplantable artificial ovarian matrix. SLM alginate is purified alginate, with 50% of the monomers being mannuronate, which will determine a biomaterial with increased flexibility and is easily degradable (35). The reality shows a reduced matrix degradation with a peripheral process of neoangiogenesis at the level periphery of the graft.

The procedure of decellularized ovarian extracellular matrix has been used to replace many heart, lung, kidney, and liver tissues. Recently, decellularized tissue has been used in 3D reconstruction with molecules from the extracellular matrix component of their native structure. The technique decellularizes the homologous tissue and recellularizes the matrix with autologous cells (36, 37).

### *Synthetic polymers*

Synthetic polymers are biocompatible, long-lasting and have good degradation, porosity and stiffness properties. Even though several essential molecules are missing, integrating biologically active substances in their structure promotes cell adhesion, differentiation, and proliferation. Instead, the degradation of these synthetic polymers releases acidic compounds that can stimulate inflammatory processes (38, 39).

Polyethylene glycol is a synthetic polymer that can be modified by crosslinking for various medical purposes, especially copolymerization, to create easily biodegradable or highly adherent matrices after cell invasion from adjacent tissue (39, 40).

## Future trends

### *Other possible sources of follicles and oocytes*

If cancer patients need to start treatment quickly and cannot perform cryopreservation of ovarian tissue, one option for preserving fertility is to use pluripotent stem cells as an alternative source of ovarian follicles. In a study realized in mice to obtain female germ cells to make an artificial ovary, the authors implanted the aggregated ovaries under the ovarian bursa and the renal capsule. Then these derived oocytes were fertilized, with a live birth rate of 3.9% (41, 42).

Studies have been directed to induced pluripotent stem cells because oocyte differentiation could not be achieved using embryonic stem cells (IPSC). The presence of epigenetic changes in induced pluripotent stem cells requires reprogramming the oocyte production process (43).

The technique of generating functional human artificial oocytes by transferring nuclear DNA derived from the somatic cells of cancer patients into donor-enucleated oocytes. Priority was then given to using IPSCs, based on excellent results in mice, where mature oocytes and healthy live offspring were obtained. This procedure was resumed after oogenesis with human IPSC cells did not exceed the oogonium stage. These procedures must perform a haploid number of chromosomes in the DNA derived from somatic cells and the reprogramming of this DNA to be ready in the implantation stage (44).

### *Reestablishment of the ovaries' endocrine function*

Premature ovarian failure (POF) affects women who survive cancer and girls who reach puberty. The consequences of ovarian endocrine failure are osteopenia, muscle hypotrophy, and cardiovascular disease. The current treatment for POF is hormone replacement therapy, which predisposes cancer (45).

David *et al*, in a study of ovariectomized mice, evaluated the restoration of ovarian endocrine function by transplanting allogeneic ovarian tissue encapsulated in alginate capsules or TheraCyte®, the latter causing follicular growth and effectively isolating the graft from identification by host immune mechanisms (46).

The study by Zhou *et al* (47) highlighted the use of a fibrin alginate network that preserves the 3D configuration of the follicle, as well as poor communication of the junction between oocytes and granular cells. The idea of an artificial ovary comes in addition to the studies conducted by Eddie *et al* and Arslan *et al* on 3D modeling of

the reproductive tract (fimbriae of the fallopian tubes and endocervix) (48, 49).

## CONCLUSIONS

The artificial ovary represents a challenge, being a viable alternative to restore fertility in cancer patients who cannot benefit from cryopreserved ovarian tissue transplantation. Another area where this artificial ovary could be used would be research into the gonadotoxicity of various treatments or the interpretation of physiological mechanisms. Thus, transplantation of human ovarian follicles does not delay the cancer treatment, does not require prior ovarian stimulation, reduces the risk of reintroducing malignant cells, and offers a real option for cancer patients. In contrast, it does not cause the resumption of ovarian endocrine function and produces a low growth of follicles and oocytes. ❑

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