Ku Seung-Yup (Orcid ID: 0000-0002-6423-854X)

A New Possibility in Fertility Preservation: the Artificial Ovary

Eun Cho[1*], Yoon Young Kim[1,2*], Kevin Noh[3], Seung-Yup Ku[1,2]

[1]Seoul National University College of Medicine, Seoul, South Korea, [2]Department of Obstetrics and Gynecology, Seoul National University Hospital, Seoul, South Korea, [3]College of Human Ecology, Cornell University, New York, USA

*These authors contributed equally to this work.

Running Title: Clinical perspectives of artificial ovary

Corresponding author: Seung-Yup Ku, MD, PhD

Department of Obstetrics and Gynecology, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 03080, South Korea.

Tel: +82-2-2072-1971, Fax: +82-2-762-3599, E-mail: jyhsyk@snu.ac.kr

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/term.2870

Abstract

Conventional fertility preservation methods such as oocyte or embryo cryopreservation are currently insufficient to treat including those patients with pre-pubertal cancer and premature ovarian failure. Ovarian tissue cryopreservation presents as an alternative but has limitations with a potential risk of reintroducing malignant cells in patients who recovers from cancer, those of chemotherapy prior to tissue cryopreservation. The so-called "artificial ovary" aims to resolve this issue by transplanting isolated follicles with or without a biological scaffold. The artificial ovary may also offer an effective alternative option for those who cannot benefit from traditional assisted reproductive techniques such as *in vitro* fertilization (IVF). To date, in animal studies and human trial, the artificial ovary restored endocrine function, achieved *in vivo* follicular development, and resulted in successful pregnancies. However, development of a technique for higher follicular recovery rate, and a more optimized design of delivery scaffold, better transplantation techniques to prevent post-surgical ischemia, and consideration for genetic safety are required for safer and consistent human clinical applications. Ideas from different transplantation surgeries (e.g. entire ovary, ovarian cortex, and transplantation with tissue-engineered products) can be applied to enhance the efficacy of artificial ovarian transplantation. For the better application of artificial ovary, a deeper understanding of mechanical and biochemical properties of the ovary and folliculogenesis after cryopreservation, transplantation with or without scaffold, as well as development of sophisticated *in vivo* imaging techniques of transplanted artificial ovary, need to precede its efficient clinical application.

Keywords: Artificial ovary, fertility preservation, tissue engineering, cryopreservation, scaffold, transplantation

Introduction

Advances in medical diagnosis and treatment for cancer have resulted in a high survival rate from malignant cancers in the current decade. Annually, the overall estimate of 1,685,210 new cases of invasive cancer are still being diagnosed in the United States; however, the 5-year cancer survival rate has increased by 20% over the past three decades (Siegel, Miller, & Jemal, 2016). There has been an increasing attention not merely on the survival from a diagnosed cancer but also on the quality of life after recovery. One of the most important topics in the counseling of cancer survivors is fertility preservation (FP).

To date, various efforts have been made to preserve ovarian function and fertility, e.g. administration of gonadotropin-releasing hormone (GnRH) agonist prior to the initiation of chemotherapy, cryopreservation of embryos, mature oocytes, or ovarian tissue (Pereira et al., 2017). Although means of ovarian protections (Abir, Fisch, Raz, Nitke, & Ben-Rafael, 1998) were attempted to prevent or decrease the loss of fertility before the chemo- or radiotherapy (Fisch & Abir, 2018), current treatments are still highly toxic to gonads such as ovary and testis. When chemotherapy impairs ovarian function, patients present reduced antral follicle counts and low anti-Müllerian hormone (AMH) levels that are indicative of a low reproductive potential (Wenners et al., 2017). Patients with premature ovarian failure (POF) due to benign diseases such as ovarian endometriomas, cysts, and pelvic inflammatory disease also have FP needs (J. Donnez & Dolmans, 2017; J. Donnez et al., 2012; J. G. Kim et al., 2004; J. J. Kim et al., 2014).

The established methods of embryo or mature oocyte cryopreservation cannot be viable options to pre-pubertal patients with cancer and to patients whose conditions require immediate start of chemotherapy (Jacques Donnez et al., 2013). Instead, *in vitro* maturation (IVM) of immature oocytes is option for those pre-pubertal patients. Although of IVM of immature

oocytes had been difficult at early phase (J. Donnez, Martinez-Madrid, et al., 2006), recently, immature oocytes could be retrieved from ovaries of pre-pubertal girls, undergo IVM and frozen (Abir et al., 2016), and many live births have been achieved from IVM (Dahan, Tan, Chung, & Son, 2016).

Ovarian tissue cryopreservation can be another option for these patients, as this method achieved over 130 clinical cases of live-births (J. Donnez & Dolmans, 2017). However, preserved ovarian tissues may contain malignant cancer cells, and this approach has a risk of putting cancer cells back into patients (Dolmans, Luyckx, Donnez, Andersen, & Greve, 2013) (Abir et al., 2014; Abir et al., 2010; Dolmans et al., 2013). A previous investigation reported that microscopic examination, polymerase chain reaction evaluation of altered DNA expression, and xenotransplantation studies of cryopreserved ovarian tissues are insufficient for detecting cancer cells and eliminating the risk (Bastings et al., 2013). An *in vitro* study using cytotoxic T cells to remove tumor cells from ovarian tissues through bispecific antibody BIS-1 attempted to resolve this problem (Schroder et al., 2004).

In recent decades, tissue and organ engineering has presented promising solutions to otherwise unsolvable medical problems (Table 1). The artificial ovary is the extension of this effort to FP. Many researchers have described the essential components and characteristics of the artificial ovary. A group of investigators maintained that the artificial ovary refers to "a temporary surrogate of the natural ovary in which isolated follicles, ovarian stromal cells and a combination of growth factors can be encapsulated together inside a biomaterial-based scaffold" (Chiti, Dolmans, Donnez, & Amorim, 2017). Another group suggested that the artificial ovary contain isolated follicles in a biomaterial, and thus is able to preserve fertility and replace hormonal functions of the ovary with a significantly decreased risk of introducing malignant cells back into patients (Soares, Saussoy, et al., 2015). A recent review described the

artificial ovary that contains primordial follicles embedded in a matrix, as one of the promising future FP techniques (Fisch & Abir, 2018).

The term "artificial ovary" was also used to describe a tissue-based hormone therapy that restores steroid hormones in patients with POF and menopausal women (David et al., 2017; Day, David, Cichon, et al., 2018; Feichtinger, Barnea, Nyachieo, Brannstrom, & Kim, 2018; Laronda et al., 2015; Sittadjody et al., 2013).

Theoretical Overview of the Artificial Ovary

The artificial ovary (Fig. 1) aims to mimic two representative functions of the ovary: female gamete production and steroid hormone release (Y. J. Kim, J. H. Shin, et al., 2017; Y. Y. Kim, Y. J. Kim, et al., 2016; Y. Y. Kim, Tamadon, & Ku, 2017).

The ovary offers a safe storage space for oocytes within follicles (Y. Y. Kim et al., 2019; Y. Y. Kim, J. W. Yun, et al., 2016; Ross, 2011; Tamadon, Park, Kim, Kang, & Ku, 2016). It releases steroid hormones after puberty, and thereby fosters follicular development and periodic oocyte release (Y. J. Kim et al., 2013; Y. J. Kim, Ku, Rosenwaks, et al., 2010; Y. J. Kim, K. E. Park, et al., 2017). Ideally and anatomically, the artificial ovary needs to contain follicles isolated from cryopreserved ovarian tissue and possibly other ovarian cells to provide growth factors (Chiti et al., 2017). It also requires a proper delivery scaffold that is biocompatible, minimally inflammatory, suitable for neo-angiogenesis, and degradable after engraftment for not to disturb follicular growth and migration (Amorim & Shikanov, 2016). Isolated follicles in a delivery scaffold are then transplanted in patients, either in an orthotopic location or in a heterotopic location (Jacques Donnez et al., 2013). After transplantation, follow-up and evaluation of its function is essential for reproductive planning and patient health.

This strategy can be more efficient than follicle transplantation only or ovarian tissue transplantation from a therapeutic perspective, as follicles are transferred instead of ovarian tissue, therefore death by ischemia could be avoided or decreased. The ideal delivery scaffold also requires modifications specific to method of cancer treatment or patient conditions.

Issues of Preserving Ovarian Tissue

A small volume of ovarian cortical tissue is sufficient for cryopreservation since most follicles reside in ovarian cortex rather than the medulla. However, it is reasonable to remove the entire ovary prior to gonadotoxic cancer treatment because it provides a sufficiently large pool of follicles even after follicular loss during the processing (Dolmans et al., 2008; J. Kim et al., 2016; Paulini et al., 2016; Smith et al., 2014). Therefore, if patients have high risks of losing fertility, cryopreserving one entire ovary can result in more satisfactory results.

The traditional cryopreservation method is the slow freezing technique, however relatively vitrification technique is used for human samples contributing to its high cell viability and minimal damage after thawing (Amorim, Curaba, Van Langendonckt, Dolmans, & Donnez, 2011; Argyle, Harper, & Davies, 2016; Y. Y. Kim et al., 2011; Silber, 2012). In terms of an entire ovary, there has been no efficient cryopreservation protocols and no clinical precedents of cryopreserved ovary transplantation (S. S. Kim, 2010). Cryopreservation of an entire ovary is challenging because of extracellular ice formation during the process, especially intravascular ice formation can destroy the organ's structure and impair its function (S. S. Kim, 2010). The cooling rate is also restricted by the organ's dimension and geometry, and it is difficult to deliver cryoprotectants evenly to all cells in a limited time (S. S. Kim, 2010). Cryotechnology for an entire organ is still being developed, thus at its current status, it is practical to cryopreserve small slices of the ovary to prevent follicular loss and damage (Liu &

Pan, 2016).

After patients recover from diseases and when they desire to have children, cryopreserved ovarian tissue can be processed to thaw and transplant as the artificial ovary. Long-term cryopreservation does not impact the quality of human ovarian tissue and therefore provides flexibility to patients to schedule their pregnancies (Fabbri et al., 2016). The time for isolating follicles, before or after cryopreservation, has not been found to influence their viability, therefore follicles can be isolated after thawing cryopreserved ovarian tissue (Vanacker et al., 2013).

Follicular Isolation and Selection

A mechanical method or collagenase treatment in addition to the mechanical treatment is widely used to isolate follicles from ovarian tissues. The collagenase treatment helps to isolate more follicles than the mechanical method alone, but causes damages follicles' basement membranes and affects the viability and development of follicles (Dong et al., 2014). A superior efficacy of liberase, a mixture of purified enzymes, was reported in regard to follicular isolation outcomes (Dolmans et al., 2006). Many steps of follicular washing during the isolation procedure have been included to decrease the risk of malignant cell inclusion (Paulini et al., 2016). However, the retrieval rate of viable follicles with intact morphology and complete granulosa cell (GC) layers is still not satisfactory. More researches are being conducted for a more effective follicular isolation technique.

After follicular isolation, the type and number of follicles to be placed in a delivery scaffold must be determined. There are follicles at different development stages in the ovary starting from primordial follicles to primary, secondary, pre-antral, and antral follicles (Hsueh,

Kawamura, Cheng, & Fauser, 2015). Grafting secondary follicles in the artificial ovary has superior outcomes in terms of a high follicular survival and growth rate than grafting primordial-primary follicles (Chiti et al., 2016). The number of follicles in a delivery scaffold should also be optimized. A delivery matrix must include an enough number of follicles to produce mature oocytes after transplantation but not too many follicles to maintain a small size of the delivery matrix. A small sized delivery matrix may be important for neovascularization and for the prevention of ischemic injury after transplantation (Revel, Laufer, Ben Meir, Lebovich, & Mitrani, 2011).

Designing Scaffolds for Isolated Follicle Delivery

Designing a scaffold to deliver isolated follicles is a crucial aspect in developing the artificial ovary. As the basic aim associated with an artificial ovary is to transplant it in a human body, the material used for producing scaffolds must meet biosafety and clinically compatible standards. Fundamental requirements include adequate protection and support to engraftment of follicles, biocompatibility, adaptability to human body temperature, degradability for follicle proliferation and migration, and vessel formation capacity to provide oxygen and nutrients to cells for successful grafting (Amorim & Shikanov, 2016; Laronda et al., 2017; Vanacker et al., 2012). A selection of a biomaterial suitable for supporting the artificial ovary from a wide range of biomaterials is challenging, however promising results have been reported in animal studies (Table 2). Natural polymers such as collagen, fibrin, plasma clot, alginate, and decellularized ovarian ECM and synthetic polymer ethylene glycol have been explored, and the artificial ovary-friendly chamber system and generation of ovary-like structure using three-dimensional printing technique has also been studied and germ line transmission was confirmed (Amorim & Shikanov, 2016; Laronda et al., 2017).

However, more standardized methods to maximize the consistent efficiency of the artificial ovary are needed. Many studies reported transplanted follicular development *in vivo* and changes in follicular stage composition after grafting, however the overall follicle recovery rate after transplantation has not fully been evaluated. A few studies reported the follicular recovery rates, which are useful in comparing the overall efficiencies of different candidate biomaterials (Paulini et al., 2016). They may help determine the number and distribution pattern of follicles in the delivery-vehicle biomaterials. The transplantation duration also influences the number of follicles to be transferred, thus time periods for performing a transplantation should be specified and standardized. A scaffold composed of fibrin gel and 15% platelet lysate had a 48.31% follicular recovery rate 14 days after transplantation (Rajabzadeh, Eimani, Mohseni Koochesfahani, Shahvardi, & Fathi, 2015), and a scaffold made of plasma clot was reported to have a 28.97% recovery rate 22 weeks after transplantation (Dolmans et al., 2008).

As securing oxygen supply via the blood vessels is crucial for a successful transplantation, the level of neoangiogenesis induced in different designs should also be comparatively analyzed. New vessel formation in transplantation sites can be compared based on the number of vessels per area or the vessel surface area per total surface area (J. Kim et al., 2016; Laronda et al., 2017; Vanacker et al., 2012). Artificial ovaries transplanted with biological scaffold that resulted in pregnancies in animal studies promoted vessel formation either when vascular endothelial growth factors (VEGF) were added to fibrin or when the gelatin scaffolds had reliable connectivity (Kniazeva et al., 2015; Laronda et al., 2017). The size and thickness of the scaffold are related to the vessel infiltration capacity as well as the number of follicles transplanted (Revel et al., 2011).

Besides growth factors, stromal cells and endothelial cells that are transplanted along with follicles may provide suitable paracrine environment for the graft and improve neovascularization (Dath et al., 2011). Ovarian stromal cells are known to differentiate into theca cells which are important for steroid hormone production and follicular development (Magoffin, 2005). A previous study compared the viability and vascular area after grafting fresh ovarian cortical cell, cryopreserved-thawed ovarian cortical cells, and fresh and cryopreserved-thawed ovarian medullary cells (Soares, Sahrari, et al., 2015). The fresh ovarian medulla was found to be the best cell source to provide an environment for paracrine secretion. In addition, local endothelial cells in ovarian tissues, rather than bone marrow-derived vascular progenitor cells, contributed to neovascularization during follicular development (Kizuka-Shibuya et al., 2014). Therefore, combined use of stromal and endothelial cells from fresh ovarian medullary tissues should be considered for co-transplantation with follicles to increase the efficiency of the artificial ovary.

Other factors such as neurotransmitter substance P (SP) and autologous mesenchymal stem cells from the bone marrow can be supplemented to enhance the efficiency of engraftment. SP was found to recruit stem cells to tissue injury sites and acted as a key molecule for tissue regeneration. It improved neovascularization when added to a transplantation material (Ko et al., 2012). Autologous mesenchymal stem cell transplantation to the ovary was reported to recover ovarian function in a patient with POF and resulted in a pregnancy (Bhartiya, Hinduja, Patel, & Bhilawadikar, 2014; Edessy et al., 2016). As in angiogenesis, effective methods to compare the degradation rates and mechanical strength for different biomaterials should be evaluated. A deeper understanding of folliculogenesis and the mechanical and biochemical properties of the ovary will augment the platform to optimize the design and development process of the artificial ovary (Amorim & Shikanov, 2016; Smith et al., 2014).

Transplantation Sites and Surgical Considerations

Artificial ovaries have been transplanted in mice at various sites such as the kidney capsule, abdominal cavity, peritoneal pocket, subcutaneous pocket, and ovarian bursa. The placement of an artificial ovary in a human can be decided based on clinical examples of ovarian tissue transplantation. The two main categories for ovarian tissue transplantation sites are orthotopic (e.g. pelvic cavity, ovary, peritoneal window) and heterotopic (e.g. forearm, neck, rectus muscle) transplantation (Jacques Donnez et al., 2013). The most apparent difference is that the heterotopic graft of the artificial ovary does not allow natural conception, while orthotopic transplantation can achieve natural pregnancy although it still requires the proper positioning of the artificial ovary (Kniazeva et al., 2015).

When heterotopic transplantation is conducted, several factors should be considered such as differences in body temperature, pressure, paracrine factors, and blood supply (Y. J. Kim, Y. Y. Kim, et al., 2017). In this context, orthotopic grafts naturally provide a favorable environment for follicular development (Jacques Donnez et al., 2013; Oktay et al., 2004). Nevertheless, heterotopic transplantation has benefits such as easier access, relatively simpler transplantation procedure, and cost-effectiveness in cases of repeated transplantations (Jacques Donnez et al., 2013). Heterotopic grafts' long-term restoration of endocrine function has been demonstrated, and a recent study reported that heterotopic grafts cause less apoptosis than orthotopic grafts (Damasio et al., 2016; S. S. Kim, 2012). More studies are needed to identify the appropriate transplantation sites, however orthotopic transplantation is deemed to satisfy the ideal concept of the artificial ovary as it allows natural ovulation and pregnancy.

The surgical procedures for artificial ovary transplantation in animal studies usually follow general surgical outlines. Briefly, under anesthesia, after incision and localization of internal structures, the artificial ovaries are placed at intended locations. When they are placed

inside the broad ligament, a small pocket is created within the ligament, and then sutured after engraftment (Kniazeva et al., 2015). If they are placed at the circular pocket created on the inner side of the peritoneum, the peritoneal surface is scratched with a scalpel blade to induce angiogenesis (Chiti et al., 2016; Luyckx et al., 2014).

The two major concerns after general transplantation surgery are immune rejection and ischemic injury. Since transplanted ovarian cells in the artificial ovary are of an autologous origin, they are free from serious immune rejection. However, immune reaction against the delivery biomaterials is still a concern, thus the scaffold should be designed to alleviate the immune response (Day, David, Kim, et al., 2018; Franz, Rammelt, Scharnweber, & Simon, 2011). In addition to immunological consideration, vessel formation after transplantation is essential to prevent ischemia and to allow hormone interaction (J. Donnez et al., 2004; Martinez-Madrid, Dolmans, Van Langendonckt, Defrere, & Donnez, 2004; Van Eyck et al., 2009).

Various attempts have been made to minimize ischemia and reperfusion injury during transplantation using animal models (Friedman et al., 2012). In addition to the use of angiogenic biomaterial or co-transplantation of endothelial cells, graft treatment with VEGFs and vitamin E, and host treatment with vitamin E and gonadotropins resulted in a better transplantation outcomes (Abir et al., 2011). Melatonin was used in animal ovary transplantation studies to enhance graft outcomes (Friedman et al., 2012) (Shiroma, Botelho, Damous, Baracat, & Soares-Jr, 2016). These trials currently remain at the animal experiment stage, but can be extended to human studies in the future.

Established concepts from the entire ovary, ovarian cortex, orthotopic ovarian tissue, and other tissue-engineered organ transplantations in humans (Table 1, 3) can be applied to further improve neovascularization in the artificial ovary. Firstly, in ovarian cortex and

orthotopic ovarian tissue transplantations, it is emphasized that implanted tissues have a small thickness (Revel et al., 2011; Silber et al., 2008; Silber & Gosden, 2007; Silber et al., 2005). Secondly, coagulation of transplantation site edge can be considered to induce angiogenesis at least 7 days before surgery (J. Donnez et al., 2004). Thirdly, the patient may be administered human recombinant erythropoietin during and after the surgery to increase (M. J. Elliott et al., 2017). Additionally, potential vessels for anastomosis can be identified and utilized as in the case of an entire ovary transplantation graft (P. Mhatre & Mhatre, 2006; Pravin Mhatre, Mhatre, Modi, & Doshi, 2003; A. Raya-Rivera et al., 2011; A. M. Raya-Rivera et al., 2014; A. M. Raya-Rivera et al.). Taken together, effective neovascularization should be achieved through anastomosis or through the addition of angiogenic factors and pre-surgical procedures.

Evaluation and Monitoring

Prior to FP procedures, ovarian reserve can be assessed by serum hormone (e.g. AMH, luteinizing hormone, follicle stimulating hormone, estradiol) level, ultrasonographic evaluation of ovarian volume and follicular count, and follicular density ovarian tissue biopsy (Belaisch-Allart, Dufetre, Allart, & De Mouzon, 1991; Choi et al., 2006; Y. J. Kim, Ku, Jee, et al., 2010; Lass et al., 1997; Schmidt et al., 2005; Wenners et al., 2017). As cryopreservation of ovarian tissue causes follicular loss, post-thaw follicular viability should be estimated through morphological evaluation and florescent staining (Carroll & Gosden, 1993; J. Donnez et al., 2004; Martinez-Madrid et al., 2004).

After transplantation of the artificial ovary in animal studies, follicular counts, histologic findings, and functionality of grafts can be examined under fluorescent or transmission electron microscope. Morphological integrity of the basement membrane, detachment between GCs and oocyte, pyknotic body, condensed chromatin, and follicular

atresia can be observed in histological evaluation (Amorim, Van Langendonckt, David, Dolmans, & Donnez, 2009; Gougeon, 1986; Rajabzadeh et al., 2015). Follicular growth and function of the graft can be evaluated by identifying the number of primordial, primary, and secondary follicles, and of corpora lutea (Myers, Britt, Wreford, Ebling, & Kerr, 2004) (Table 4).

TUNEL assay for DNA fragmentation and apoptosis (Chiti et al., 2016; Vanacker et al., 2012), and Ki-67 staining for proliferation (Dolmans et al., 2007; Luyckx et al., 2013) helps assess follicular cell viability and growth. The expression of P450 aromatase and FSHR on GCs (Kossowska-Tomaszczuk et al., 2010) can be used as well. Neo-angiogenesis in retrieved graft tissues can be evaluated through microscopic imaging and counting the number of vessels per area, and through immunostaining for platelet endothelial cell adhesion molecules, platelet derived growth factor receptor-β1, and VEGFs (Laronda et al., 2017; Nisolle, Casanas-Roux, Qu, Motta, & Donnez, 2000; Telfer, Torrance, & Gosden, 1990).

Electron paramagnetic resonance imaging allows *in vivo* evaluation of implant oxygenation (Van Eyck et al., 2009). Inflammatory responses can be evaluated through CD45 and F4/80 immunostaining (Laronda et al., 2015; Vanacker, Dolmans, Luyckx, Donnez, & Amorim, 2014). Ovarian functional recovery in mice can be validated with lowered serum FSH concentration, elevated estrogen level, increased inhibin level, and pregnancy (Carroll & Gosden, 1993; J. Kim et al., 2016; Y. J. Kim, K. E. Park, et al., 2017; Y. Y. Kim, H. Kim, et al., 2016; Laronda et al., 2015; Laronda et al., 2017; Smith et al., 2014).

When the artificial ovary is to be applied in a clinical setting (Table 3), the transplanted graft may be followed up and evaluated by observing inflammatory immune responses (e.g. CRP level, CD4/WBC count), measuring the serum hormone level, following the menstrual cycle, and imaging follicles via ultrasonographic evaluation (J. Donnez et al., 2004; J. Donnez,

Martinez-Madrid, et al., 2006; Meirow et al., 2005; P. Mhatre & Mhatre, 2006; Pravin Mhatre et al., 2003; Povoa et al., 2016; Silber et al., 2005). Moreover, it is important to confirm that genetic information of oocytes within follicles is not altered during the FP process. A genetic test on oocytes before transplantation and after *in vivo* maturation and induction may be necessary.

Furthermore, it would be ideal to observe how the transplanted artificial ovary establishes on the recipient. Molecular imaging techniques such as PET can be useful in detecting any abnormalities in graft if appropriate biomarkers are identified (Bailly et al., 2017). A previous study reported that 18F-FDG PET/CT detected an infection in the implanted intracardiac device (Dejust, Guedec-Ghelfi, Blanc-Autrant, Lepers, & Morland, 2017). Besides PET/CT, near infrared spectroscopy and hyperspectral imaging may enable oxygenation monitoring of the transplanted tissue *in vivo* early enough to predict functionality (Holmer et al., 2016; Jarraya, Mohamed, Sofiene, & Kolsi, 2016; Manley, 2014). An intravital microscopy that allows real-time *in vivo* observation of the vessels at the cellular level can also be adapted to monitor neo-angiogenesis in the graft (Fisher et al., 2016; Pang et al., 2015).

Social and Bioethical Issues

The artificial ovary aims to assist FP in female patients who undergo gonadotoxic treatments or POF, however similar to other technical advances, it cannot evade ethical issues except for autologous transplantation. First of all, Limited availability due to relatively defined number of ovarian follicles and their characteristics as germ cells having individual genetic information may raise bioethical concerns. Thus, the artificial ovary challenges justice in the distribution of donated organs. The distribution issue of FP techniques was recently covered by a recent report (Cardozo, Huber, Stuckey, & Alvero, 2017).

Fertility preservation issue is associated with financial coverage by social insurance system. For instance, only Connecticut and Rhode Island in the United States support insurance coverage for FP after chemo- or radiotherapy. As a result, a very small percentage (4-10%) of patients with breast cancer pursue FP because of its great financial burden (Cardozo et al., 2017). They also argued that fertility loss should be viewed as a "life-affirming" iatrogenic condition and be covered by insurance (Cardozo et al., 2017). The availability of the artificial ovary and its presumably high cost at future clinics bring up social concerns in its distribution similar to other FP techniques.

The potential negative social influences and psychological sense of deprivation in patients who are unable to afford artificial ovaries cannot be ignored (Hansson, 2005). Nonetheless, as in the Republic of Korea and some European countries where a low yearly birth rate is a serious social issue, governments may provide medical insurance or subsidy for the expenditures required to foster childbearing, possibly including the artificial ovary.

Another bioethical concern rises from the nature of handling germ cells. Legislation in many countries prohibits genetic germline manipulation. The United Kingdom's Human Fertilization and Embryology Act states that "no person shall place in a woman any gametes other than permitted egg" in which nuclear and mitochondrial DNA has not been altered ("Human Fertilisation and Embryology Act 1990," 1990). The artificial ovary is yet to be tested in human subjects, and thus requires careful planning to avoid DNA mutations. A failure to demonstrate genetic and physiological safety will trigger both scientific and moral concerns regarding the artificial ovary.

These issues become more complicated if the artificial ovary extends its potential usage to individuals who do not undertake cancer treatments. The artificial ovary can become a method to delay childbearing for career or personal reasons, and a genetic manipulation tool

for eugenic purposes (Ishii, 2017). This expected concern is conceivable considering rigorous bioethical debates on preimplantation diagnosis and genome engineering via the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 technology, as the preimplantation genetic diagnosis (PGD) is adopted in clinics and researchers recently succeeded in applying the CRISPR/Cas9 technology to edit a human embryo (Frati et al., 2017; Ma et al., 2017; Vassena et al., 2016). The artificial ovary may offer an opportunity for PGD and CRISPR genome editing when follicles are isolated and processed, and therefore lies on the extension of related bioethical controversies (Watson, Alyea, Cunningham, & Jeng, 2010).

Medical costs, in general, have a wide range of differences among countries, hospitals, services, and insurance coverage, thus it is difficult to predict the cost for the artificial ovary. Nonetheless, since its procedures are similar to that of *in vitro* fertilization (IVF), its cost can be approximated by parallel comparison. An approximate total cost of the artificial ovary is currently estimated as $5,000 higher than that of IVF. The expected cost is quite high to be recommended to all female patients undergoing chemotherapy, as cost is one of the main factors that discourage to opt for FP (Jones et al., 2017). In the future, establishing standardized mass production of the artificial ovary can become a strategic issue to lower its cost, which can in turn benefit more patients with clinical needs.

Future Perspectives

In patients with POF with follicular atresia and only few viable follicles, either donated oocytes or alternative sources for oocytes are necessary. As alternative sources for gametes, stem cells including induced pluripotent stem cells (iPSCs) have been explored. Hayashi *et al.* reported of one such alternative involving female primordial germ cell-like cells derived from embryonic stem cells or transformed from iPSCs (Hayashi et al., 2012). Mice transplanted with

these cells were able to successfully reproduce through IVM and fertilization. Nevertheless, this method is yet to be tested with human stem cells. Implementation with human stem cells should be dealt with caution, because iPSCs are known to have mitochondrial DNA mutations even when isolated from healthy donors (Prigione et al., 2011).

Oogonial stem cells (OSCs) and very small embryonic like (VSEL) stem cells in the adult mammalian ovary present as other potential sources for oocytes (Bhartiya et al., 2014; White et al., 2012). However, the use of OSCs and VSEL stem cells is still being debated upon, and more reproducible studies have to be conducted to assess their usefulness and efficacy in this regard. Table 5 summarizes the advantages and disadvantages of different sources for gametes used for transplantable artificial ovary (Bhartiya et al., 2014; Hanna & Hennebold, 2014; R. Daniel Beauchamp, 2016). Despite the risk of transmitting remaining or undiagnosed cancer cells, patients' own follicles from cryopreserved ovarian tissues can be the source for gametes in the near future, because no immunological, mutational, and ethical complications are associated with them in contrast to other sources.

Furthermore, environmental conditions can be controlled to foster interaction between granulosa cells and oocytes, and follicular development. There are reports that kit ligand and anti-kit antibody (Reynaud, Cortvrindt, Smitz, & Driancourt, 2000), and microRNAs in granulosa cells (Y. J. Kim et al., 2013; Y. Y. Kim, H. Min, et al., 2017) affect oocyte maturation and follicle development *in vitro.* Their effects in humans *in vivo* are yet to be proven (Jacques Donnez et al., 2013; Ku et al., 2002), and these genetic and signaling manipulations require caution to be exercised while handling germ cells (S. M. Kim et al., 2008; Lee et al., 2003). Yet, manipulation of these conditions can potentially be used to enhance follicular development *in vivo* (Y.Y. Kim, et al., 2018; Y.J. Kim, et al., 2018).

Conclusions

Advances in tissue and organ engineering have opened up another FP option in female patients. Animal studies on the artificial ovary achieved few pregnancies; yet, a more optimized and standardized protocol is required for clinical applications in humans. In addition to improvements in the construction of an artificial ovary, such as a better follicular isolation technique and surgical vascularization technique, a progress in other related fields will foster the development of an effective artificial ovary. A deeper understanding of folliculogenesis and the mechanical and biochemical properties of the ovary and development of standardized methods to compare the properties of different bio-scaffolds in a more objective and quantitative manner can help identify an effective biomaterial to deliver follicles. Development of a better imaging technique *in vivo* to observe vascularization and degradation of an implanted material will allow researchers and clinicians to monitor and evaluate the transplanted artificial ovary effectively. If clinically applied in the future, the artificial ovary will prove to be another milestone in the field of tissue and organ engineering.

Acknowledgements

The authors would like to appreciate the sincere assistance of Seri Kwon and Ji Won Jeong. This study was supported by grants of Ministry of Science, Technology and ICT (2016R1E1A1A01943455), and by the Medical Research Program at Seoul National University College of Medicine.

Conflicts of Interests

The authors have declared that there is no conflict of interest.

References

Abir, R., Aviram, A., Feinmesser, M., Stein, J., Yaniv, I., Parnes, D., . . . Fisch, B. (2014). Ovarian minimal residual disease in chronic myeloid leukaemia. *Reprod Biomed Online, 28*(2), 255-260. doi:10.1016/j.rbmo.2013.10.011

Abir, R., Ben-Aharon, I., Garor, R., Yaniv, I., Ash, S., Stemmer, S. M., . . . Fisch, B. (2016). Cryopreservation of in vitro matured oocytes in addition to ovarian tissue freezing for fertility preservation in paediatric female cancer patients before and after cancer therapy. *Hum Reprod, 31*(4), 750-762. doi:10.1093/humrep/dew007

Abir, R., Feinmesser, M., Yaniv, I., Fisch, B., Cohen, I. J., Ben-Haroush, A., . . . Avigad, S. (2010). Occasional involvement of the ovary in Ewing sarcoma. *Hum Reprod, 25*(7), 1708-1712. doi:10.1093/humrep/deq121

Abir, R., Fisch, B., Jessel, S., Felz, C., Ben-Haroush, A., & Orvieto, R. (2011). Improving posttransplantation survival of human ovarian tissue by treating the host and graft. *Fertil Steril, 95*(4), 1205-1210. doi:10.1016/j.fertnstert.2010.07.1082

Abir, R., Fisch, B., Raz, A., Nitke, S., & Ben-Rafael, Z. (1998). Preservation of fertility in women undergoing chemotherapy: current approach and future prospects. *J Assist Reprod Genet, 15*(8), 469-477.

Adachi, N., Ochi, M., Deie, M., Nakamae, A., Kamei, G., Uchio, Y., & Iwasa, J. (2014). Implantation of tissue-engineered cartilage-like tissue for the treatment for full-thickness cartilage defects of the knee. *Knee Surg Sports Traumatol Arthrosc, 22*(6), 1241-1248. doi:10.1007/s00167-013-2521-0

Amorim, C. A., Curaba, M., Van Langendonckt, A., Dolmans, M. M., & Donnez, J. (2011). Vitrification as an alternative means of cryopreserving ovarian tissue. *Reprod Biomed Online, 23*(2), 160-186. doi:10.1016/j.rbmo.2011.04.005

Amorim, C. A., & Shikanov, A. (2016). The artificial ovary: current status and future perspectives. *Future Oncol, 12*(20), 2323-2332. doi:10.2217/fon-2016-0202

Amorim, C. A., Van Langendonckt, A., David, A., Dolmans, M. M., & Donnez, J. (2009). Survival of human pre-antral follicles after cryopreservation of ovarian tissue, follicular isolation and in vitro culture in a calcium alginate matrix. *Human Reproduction, 24*(1), 92-99. doi:10.1093/humrep/den343

Argyle, C. E., Harper, J. C., & Davies, M. C. (2016). Oocyte cryopreservation: where are we now? *Hum Reprod Update, 22*(4), 440-449. doi:10.1093/humupd/dmw007

Atala, A., Bauer, S. B., Soker, S., Yoo, J. J., & Retik, A. B. Tissue-engineered autologous bladders for patients needing cystoplasty. *The Lancet, 367*(9518), 1241-1246. doi:10.1016/S0140-6736(06)68438-9

Bailly, C., Cléry, P.-F., Faivre-Chauvet, A., Bourgeois, M., Guérard, F., Haddad, F., . . . Bodet-Milin, C. (2017). Immuno-PET for Clinical Theranostic Approaches. *International Journal of Molecular Sciences, 18*(1), 57. doi:10.3390/ijms18010057

Bastings, L., Beerendonk, C. C., Westphal, J. R., Massuger, L. F., Kaal, S. E., van Leeuwen, F. E., . . . Peek, R. (2013). Autotransplantation of cryopreserved ovarian tissue in cancer survivors and the risk of reintroducing malignancy: a systematic review. *Hum Reprod Update, 19*(5), 483-506. doi:10.1093/humupd/dmt020

Belaisch-Allart, J., Dufetre, C., Allart, J. P., & De Mouzon, J. (1991). Comparison of transvaginal and transabdominal ultrasound for monitoring follicular development in an in-vitro fertilization programme. *Human Reproduction, 6*(5), 688-689.

Bhartiya, D., Hinduja, I., Patel, H., & Bhilawadikar, R. (2014). Making gametes from pluripotent stem cells--a promising role for very small embryonic-like stem cells. *Reprod Biol Endocrinol, 12*, 114. doi:10.1186/1477-7827-12-114

Bivalacqua, T., Steinberg, G., Smith, N., Lerner, S., Bochner, B., Lee, C., . . . Schoenberg, M. 178 Pre-clinical and clinical translation of a tissue engineered neo–urinary conduit using adipose derived smooth muscle cells for urinary reconstruction. *European Urology Supplements, 13*(1), e178. doi:10.1016/S1569-9056(14)60177-9

Bivalacqua, T., Steinberg, G., Smith, N., Lerner, S., Bochner, B., Lee, C., . . . Schoenberg, M. MP61-16 pre-clinical and clinical translation of a tissue engineered neourinary conduit using adipose derived smooth muscle cells for urinary reconstruction. *J Urol, 191*(4), e689. doi:10.1016/j.juro.2014.02.1893

Bobba, S., Chow, S., Watson, S., & Di Girolamo, N. (2015). Clinical outcomes of xeno-free expansion and transplantation of autologous ocular surface epithelial stem cells via contact lens delivery: a prospective case series. *Stem Cell Res Ther, 6*, 23. doi:10.1186/s13287-015-0009-1

Burillon, C., Huot, L., Justin, V., Nataf, S., Chapuis, F., Decullier, E., & Damour, O. (2012). Cultured autologous oral mucosal epithelial cell sheet (CAOMECS) transplantation for the treatment of corneal limbal epithelial stem cell deficiency. *Invest Ophthalmol Vis Sci, 53*(3), 1325-1331. doi:10.1167/iovs.11-7744

Cardozo, E. R., Huber, W. J., Stuckey, A. R., & Alvero, R. J. (2017). Mandating Coverage for Fertility Preservation - A Step in the Right Direction. *N Engl J Med, 377*(17), 1607-1609. doi:10.1056/NEJMp1709585

Carroll, J., & Gosden, R. G. (1993). Transplantation of Frozen - Thawed Mouse Primordial Follicles. *Human Reproduction, 8*(8), 1163-1167.

Chen, H. C., Chen, H. L., Lai, J. Y., Chen, C. C., Tsai, Y. J., Kuo, M. T., . . . Ma, D. H. (2009). Persistence of transplanted oral mucosal epithelial cells in human cornea. *Invest Ophthalmol Vis Sci, 50*(10), 4660-4668. doi:10.1167/iovs.09-3377

Chiti, M. C., Dolmans, M. M., Donnez, J., & Amorim, C. A. (2017). Fibrin in Reproductive Tissue Engineering: A Review on Its Application as a Biomaterial for Fertility Preservation. *Annals of Biomedical Engineering, 45*(7), 1650-1663. doi:10.1007/s10439-017-1817-5

Chiti, M. C., Dolmans, M. M., Orellana, R., Soares, M., Paulini, F., Donnez, J., & Amorim, C. A. (2016). Influence of follicle stage on artificial ovary outcome using fibrin as a matrix. *Human Reproduction, 31*(2), 427-435. doi:10.1093/humrep/dev299

Choi, Y. S., Ku, S. Y., Jee, B. C., Suh, C. S., Choi, Y. M., Kim, J. G., . . . Kim, S. H. (2006). Comparison of follicular fluid IGF-I, IGF-II, IGFBP-3, IGFBP-4 and PAPP-A concentrations and their ratios between GnRH agonist and GnRH antagonist protocols for controlled ovarian stimulation in IVF-embryo transfer patients. *Hum Reprod, 21*(8), 2015-2021. doi:10.1093/humrep/del091

Dahan, M. H., Tan, S. L., Chung, J., & Son, W. Y. (2016). Clinical definition paper on in vitro maturation of human oocytes. *Hum Reprod, 31*(7), 1383-1386. doi:10.1093/humrep/dew109

Damasio, L. C., Soares-Junior, J. M., Iavelberg, J., Maciel, G. A., de Jesus Simoes, M., Dos Santos Simoes, R., . . . Baracat, E. C. (2016). Heterotopic ovarian transplantation results in less apoptosis than orthotopic transplantation in a minipig model. *J Ovarian Res, 9*, 14. doi:10.1186/s13048-016-0223-1

Dath, C., Dethy, A., Van Langendonckt, A., Van Eyck, A. S., Amorim, C. A., Luyckx, V., . . . Dolmans, M. M. (2011). Endothelial cells are essential for ovarian stromal tissue

restructuring after xenotransplantation of isolated ovarian stromal cells. *Human Reproduction, 26*(6), 1431-1439. doi:10.1093/humrep/der073

David, A., Day, J. R., Cichon, A. L., Lefferts, A., Cascalho, M., & Shikanov, A. (2017). Restoring Ovarian Endocrine Function with Encapsulated Ovarian Allograft in Immune Competent Mice. *Ann Biomed Eng, 45*(7), 1685-1696. doi:10.1007/s10439-016-1780-6

Day, J. R., David, A., Cichon, A. L., Kulkarni, T., Cascalho, M., & Shikanov, A. (2018). Immunoisolating poly(ethylene glycol) based capsules support ovarian tissue survival to restore endocrine function. *J Biomed Mater Res A, 106*(5), 1381-1389. doi:10.1002/jbm.a.36338

Day, J. R., David, A., Kim, J., Farkash, E. A., Cascalho, M., Milasinovic, N., & Shikanov, A. (2018). The impact of functional groups of poly(ethylene glycol) macromers on the physical properties of photo-polymerized hydrogels and the local inflammatory response in the host. *Acta Biomater, 67*, 42-52. doi:10.1016/j.actbio.2017.12.007

De Luca, M., Albanese, E., Bondanza, S., Megna, M., Ugozzoli, L., Molina, F., . . . et al. (1989). Multicentre experience in the treatment of burns with autologous and allogenic cultured epithelium, fresh or preserved in a frozen state. *Burns, 15*(5), 303-309.

Dejust, S., Guedec-Ghelfi, R., Blanc-Autrant, E., Lepers, Y., & Morland, D. (2017). Infection of Ventricular Assist Device Detected and Monitored by 18F-FDG PET/CT. *Clin Nucl Med, 42*(9), 695-696. doi:10.1097/rlu.0000000000001736

Di Girolamo, N., Bosch, M., Zamora, K., Coroneo, M. T., Wakefield, D., & Watson, S. L. (2009). A contact lens-based technique for expansion and transplantation of autologous epithelial progenitors for ocular surface reconstruction. *Transplantation, 87*(10), 1571-1578. doi:10.1097/TP.0b013e3181a4bbf2

Dobrowolski, D., Orzechowska-Wylegala, B., Wowra, B., Wroblewska-Czajka, E., Grolik, M., Szczubialka, K., . . . Aragona, P. (2015). Cultivated Oral Mucosa Epithelium in Ocular Surface Reconstruction in Aniridia Patients. *Biomed Res Int, 2015*, 281870. doi:10.1155/2015/281870

Dolmans, M. M., Luyckx, V., Donnez, J., Andersen, C. Y., & Greve, T. (2013). Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. *Fertil Steril, 99*(6), 1514-1522. doi:10.1016/j.fertnstert.2013.03.027

Dolmans, M. M., Martinez-Madrid, B., Gadisseux, E., Guiot, Y., Yuan, W. Y., Torre, A., . . . Donnez, J. (2007). Short-term transplantation of isolated human ovarian follicles and cortical tissue into nude mice. *Reproduction, 134*(2), 253-262. doi:10.1530/rep-07-0131

Dolmans, M. M., Michaux, N., Camboni, A., Martinez-Madrid, B., Van Langendonckt, A., Nottola, S. A., & Donnez, J. (2006). Evaluation of Liberase, a purified enzyme blend, for the isolation of human primordial and primary ovarian follicles. *Human Reproduction, 21*(2), 413-420. doi:10.1093/humrep/dei320

Dolmans, M. M., Yuan, W. Y., Camboni, A., Torre, A., Van Langendonckt, A., Martinez-Madrid, B., & Donnez, J. (2008). Development of antral follicles after xenografting of isolated small human preantral follicles. *Reprod Biomed Online, 16*(5), 705-711.

Dong, F. L., Ma, L., Shi, S. L., Dai, S. J., Liu, X. G., Su, Y. C., . . . Sun, Y. P. (2014). An research on the isolation methods of frozen-thawed human ovarian preantral follicles. *Int J Clin Exp Med, 7*(8), 2298-2303.

Donnez, J., Dolmans, M.-M., Pellicer, A., Diaz-Garcia, C., Sanchez Serrano, M., Schmidt, K. T., . . . Andersen, C. Y. (2013). Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. *Fertil Steril, 99*(6), 1503-1513. doi:https://doi.org/10.1016/j.fertnstert.2013.03.030

Donnez, J., & Dolmans, M. M. (2017). Fertility Preservation in Women. *N Engl J Med, 377*(17), 1657-1665. doi:10.1056/NEJMra1614676

Donnez, J., Dolmans, M. M., Demylle, D., Jadoul, P., Pirard, C., Squifflet, J., . . . van Langendonckt, A. (2004). Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet, 364*(9443), 1405-1410. doi:10.1016/s0140-6736(04)17222-x

Donnez, J., Dolmans, M. M., Demylle, D., Jadoul, P., Pirard, C., Squifflet, J., . . . Van Langendonckt, A. (2006). Restoration of ovarian function after orthotopic (intraovarian and periovarian) transplantation of cryopreserved ovarian tissue in a woman treated by bone marrow transplantation for sickle cell anaemia: case report. *Human Reproduction, 21*(1), 183-188. doi:10.1093/humrep/dei268

Donnez, J., Jadoul, P., Pirard, C., Hutchings, G., Demylle, D., Squifflet, J., . . . Dolmans, M. M. (2012). Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. *Fertil Steril, 98*(3), 720-725. doi:10.1016/j.fertnstert.2012.05.017

Donnez, J., Martinez-Madrid, B., Jadoul, P., Van Langendonckt, A., Demylle, D., & Dolmans, M. M. (2006). Ovarian tissue cryopreservation and transplantation: a review. *Hum Reprod Update, 12*(5), 519-535. doi:10.1093/humupd/dml032

Dua, K. S., Hogan, W. J., Aadam, A. A., & Gasparri, M. In-vivo oesophageal regeneration in a human being by use of a non-biological scaffold and extracellular matrix. *The Lancet, 388*(10039), 55-61. doi:10.1016/S0140-6736(15)01036-3

Dufrane, D., Docquier, P. L., Delloye, C., Poirel, H. A., Andre, W., & Aouassar, N. (2015). Scaffold-free Three-dimensional Graft From Autologous Adipose-derived Stem Cells for Large Bone Defect Reconstruction: Clinical Proof of Concept. *Medicine (Baltimore), 94*(50), e2220. doi:10.1097/md.0000000000002220

Edessy, M., Hosni, H., Shady, Y., Waf, Y., Bakr, S., & Kamel, M. (2016). Autologous stem cells therapy, The first baby of idiopathic premature ovarian failure. *Acta Medica International, 3*(1), 19-23. doi:10.5530/ami.2016.1.7

el-Kassaby, A., AbouShwareb, T., & Atala, A. (2008). Randomized comparative study between buccal mucosal and acellular bladder matrix grafts in complex anterior urethral strictures. *J Urol, 179*(4), 1432-1436. doi:10.1016/j.juro.2007.11.101

El-Kassaby, A. W., Retik, A. B., Yoo, J. J., & Atala, A. (2003). Urethral stricture repair with an off-the-shelf collagen matrix. *J Urol, 169*(1), 170-173; discussion 173. doi:10.1097/01.ju.0000040520.75572.54

Elliott, M. J., Butler, C. R., Varanou-Jenkins, A., Partington, L., Carvalho, C., Samuel, E., . . . Birchall, M. A. (2017). Tracheal Replacement Therapy with a Stem Cell-Seeded Graft: Lessons from Compassionate Use Application of a GMP-Compliant Tissue-Engineered Medicine. *Stem Cells Transl Med, 6*(6), 1458-1464. doi:10.1002/sctm.16-0443

Elliott, M. J., De Coppi, P., Speggiorin, S., Roebuck, D., Butler, C. R., Samuel, E., . . . Birchall, M. A. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. *The Lancet, 380*(9846), 994-1000. doi:10.1016/S0140-6736(12)60737-5

Fabbri, R., Macciocca, M., Vicenti, R., Pasquinelli, G., Caprara, G., Valente, S., . . . Paradisi, R. (2016). Long-term storage does not impact the quality of cryopreserved human ovarian tissue. *J Ovarian Res, 9*(1), 50. doi:10.1186/s13048-016-0261-8

Feichtinger, M., Barnea, E. R., Nyachieo, A., Brannstrom, M., & Kim, S. S. (2018). Allogeneic ovarian transplantation using immunomodulator preimplantation factor (PIF) as monotherapy restored ovarian function in olive baboon. *J Assist Reprod Genet, 35*(1), 81-89. doi:10.1007/s10815-017-1051-y

Fisch, B., & Abir, R. (2018). Female fertility preservation: past, present and future. *Reproduction, 156*(1), F11-F27. doi:10.1530/REP-17-0483

Fisher, D. T., Muhitch, J. B., Kim, M., Doyen, K. C., Bogner, P. N., Evans, S. S., & Skitzki, J. J. (2016). Intraoperative intravital microscopy permits the study of human tumour vessels. *Nat Commun, 7*, 10684. doi:10.1038/ncomms10684

Franz, S., Rammelt, S., Scharnweber, D., & Simon, J. C. (2011). Immune responses to implants - a review of the implications for the design of immunomodulatory biomaterials. *Biomaterials, 32*(28), 6692-6709. doi:10.1016/j.biomaterials.2011.05.078

Frati, P., Fineschi, V., Di Sanzo, M., La Russa, R., Scopetti, M., Severi, F. M., & Turillazzi, E. (2017). Preimplantation and prenatal diagnosis, wrongful birth and wrongful life: a global view of bioethical and legal controversies. *Hum Reprod Update, 23*(3), 338-357. doi:10.1093/humupd/dmx002

Friedman, O., Orvieto, R., Fisch, B., Felz, C., Freud, E., Ben-Haroush, A., & Abir, R. (2012). Possible improvements in human ovarian grafting by various host and graft treatments. *Hum Reprod, 27*(2), 474-482. doi:10.1093/humrep/der385

Fulco, I., Miot, S., Haug, M. D., Barbero, A., Wixmerten, A., Feliciano, S., . . . Martin, I. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. *The Lancet, 384*(9940), 337-346. doi:10.1016/S0140-6736(14)60544-4

Gallico, G. G., 3rd, O'Connor, N. E., Compton, C. C., Kehinde, O., & Green, H. (1984). Permanent coverage of large burn wounds with autologous cultured human epithelium. *N Engl J Med, 311*(7), 448-451. doi:10.1056/nejm198408163110706

Gonfiotti, A., Jaus, M. O., Barale, D., Baiguera, S., Comin, C., Lavorini, F., . . . Macchiarini, P. The first tissue-engineered airway transplantation: 5-year follow-up results. *The Lancet, 383*(9913), 238-244. doi:10.1016/S0140-6736(13)62033-4

Gosden, R. G. (1990). Restitution of fertility in sterilized mice by transferring primordial ovarian follicles. *Human Reproduction, 5*(2), 117-122.

Gougeon, A. (1986). Dynamics of follicular growth in the human: a model from preliminary results. *Human Reproduction, 1*(2), 81-87.

Guo, Q., Pi, Y., Dong, Y., & Zhu, J. (2013). Tissue-engineered epithelium transplantation for severe ocular surface burns. *Eye Sci, 28*(1), 24-29.

Hamilton, N. J., Kanani, M., Roebuck, D. J., Hewitt, R. J., Cetto, R., Culme-Seymour, E. J., . . . Birchall, M. A. (2015). Tissue-Engineered Tracheal Replacement in a Child: A 4-Year Follow-Up Study. *Am J Transplant, 15*(10), 2750-2757. doi:10.1111/ajt.13318

Hanna, C. B., & Hennebold, J. D. (2014). Ovarian germline stem cells: an unlimited source of oocytes? *Fertil Steril, 101*(1), 20-30. doi:10.1016/j.fertnstert.2013.11.009

Hansbrough, J. F., Mozingo, D. W., Kealey, G. P., Davis, M., Gidner, A., & Gentzkow, G. D. (1997). Clinical trials of a biosynthetic temporary skin replacement, Dermagraft-Transitional Covering, compared with cryopreserved human cadaver skin for

temporary coverage of excised burn wounds. *J Burn Care Rehabil, 18*(1 Pt 1), 43-51.
Hansson, S. O. (2005). Implant ethics. *J Med Ethics, 31*(9), 519-525. doi:10.1136/jme.2004.009803
Hayashi, K., Ogushi, S., Kurimoto, K., Shimamoto, S., Ohta, H., & Saitou, M. (2012). Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. *Science, 338*(6109), 971-975. doi:10.1126/science.1226889
Hibino, N., McGillicuddy, E., Matsumura, G., Ichihara, Y., Naito, Y., Breuer, C., & Shinoka, T. (2010). Late-term results of tissue-engineered vascular grafts in humans. *J Thorac Cardiovasc Surg, 139*(2), 431-436, 436.e431-432. doi:10.1016/j.jtcvs.2009.09.057
Hohlfeld, J., de Buys Roessingh, A., Hirt-Burri, N., Chaubert, P., Gerber, S., Scaletta, C., . . . Applegate, L. A. (2005). Tissue engineered fetal skin constructs for paediatric burns. *Lancet, 366*(9488), 840-842. doi:10.1016/s0140-6736(05)67107-3
Holmer, A., Tetschke, F., Marotz, J., Malberg, H., Markgraf, W., Thiele, C., & Kulcke, A. (2016). Oxygenation and perfusion monitoring with a hyperspectral camera system for chemical based tissue analysis of skin and organs. *Physiol Meas, 37*(11), 2064-2078. doi:10.1088/0967-3334/37/11/2064
Hou, T., Wu, Z., Xing, J., Wu, X., Luo, F., Xie, Z., . . . Xu, J. (2016). Tissue-engineered bone treating simple bone cyst--a new strategy. *J Surg Res, 200*(2), 544-551. doi:10.1016/j.jss.2015.09.032
Hsueh, A. J., Kawamura, K., Cheng, Y., & Fauser, B. C. (2015). Intraovarian control of early folliculogenesis. *Endocr Rev, 36*(1), 1-24. doi:10.1210/er.2014-1020 10.1210/er.2015.36.issue-1.edboard
Human Fertilisation and Embryology Act 1990. (1990). *Halsburys Statut Engl G B, 38*, 105-161.
Ishii, T. (2017). Germ line genome editing in clinics: the approaches, objectives and global society. *Brief Funct Genomics, 16*(1), 46-56. doi:10.1093/bfgp/elv053
Izumi, K., Neiva, R. F., & Feinberg, S. E. (2013). Intraoral grafting of tissue-engineered human oral mucosa. *Int J Oral Maxillofac Implants, 28*(5), e295-303. doi:10.11607/jomi.te11
Jarraya, A., Mohamed, S., Sofiene, L., & Kolsi, K. (2016). Near-infrared spectrometry in pregnancy: progress and perspectives, a review of literature. *Pan Afr Med J, 23*, 39. doi:10.11604/pamj.2016.23.39.5857
Jones, G., Hughes, J., Mahmoodi, N., Smith, E., Skull, J., & Ledger, W. (2017). What factors hinder the decision-making process for women with cancer and contemplating fertility preservation treatment? *Hum Reprod Update, 23*(4), 433-457. doi:10.1093/humupd/dmx009
Jungebluth, P., Alici, E., Baiguera, S., Blomberg, P., Bozóky, B., Crowley, C., . . . Macchiarini, P. Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study. *The Lancet, 378*(9808), 1997-2004. doi:10.1016/S0140-6736(11)61715-7
Kaigler, D., Avila-Ortiz, G., Travan, S., Taut, A. D., Padial-Molina, M., Rudek, I., . . . Giannobile, W. V. (2015). Bone Engineering of Maxillary Sinus Bone Deficiencies Using Enriched CD90+ Stem Cell Therapy: A Randomized Clinical Trial. *J Bone Miner Res, 30*(7), 1206-1216. doi:10.1002/jbmr.2464
Kates, M., Singh, A., Matsui, H., Steinberg, G. D., Smith, N. D., Schoenberg, M. P., & Bivalacqua, T. J. (2015). Tissue-engineered urinary conduits. *Curr Urol Rep, 16*(3), 8. doi:10.1007/s11934-015-0480-3

Kim, J., Perez, A. S., Claflin, J., David, A., Zhou, H., & Shikanov, A. (2016). Synthetic hydrogel supports the function and regeneration of artificial ovarian tissue in mice. *NPJ Regen Med, 1*. doi:10.1038/npjregenmed.2016.10

Kim, J. G., Kim, H., Ku, S. Y., Kim, S. H., Choi, Y. M., & Moon, S. Y. (2004). Association between human alpha 2-Heremans Schmidt glycoprotein (AHSG) polymorphism and endometriosis in Korean women. *Fertil Steril, 82*(6), 1497-1500. doi:10.1016/j.fertnstert.2004.05.089

Kim, J. J., Choi, Y. M., Chae, S. J., Hwang, K. R., Yoon, S. H., Kim, M. J., . . . Kim, J. G. (2014). Vitamin D deficiency in women with polycystic ovary syndrome. *Clin Exp Reprod Med, 41*(2), 80-85. doi:10.5653/cerm.2014.41.2.80

Kim, S. M., Kim, S. H., Lee, J. R., Jee, B. C., Ku, S. Y., Suh, C. S., . . . Moon, S. Y. (2008). Association of leptin receptor polymorphisms Lys109Arg and Gln223Arg with serum leptin profile and bone mineral density in Korean women. *Am J Obstet Gynecol, 198*(4), 421 e421-428. doi:10.1016/j.ajog.2007.10.799

Kim, S. S. (2010). Time to re-think: ovarian tissue transplantation versus whole ovary transplantation. *Reprod Biomed Online, 20*(2), 171-174. doi:10.1016/j.rbmo.2009.11.019

Kim, S. S. (2012). Assessment of long term endocrine function after transplantation of frozen-thawed human ovarian tissue to the heterotopic site: 10 year longitudinal follow-up study. *J Assist Reprod Genet, 29*(6), 489-493. doi:10.1007/s10815-012-9757-3

Kim, Y. J., Kim, Y. Y., Kang, B. C., Kim, M. S., Ko, I. K., Liu, H. C., . . . Ku, S. Y. (2017). Induction of multiple ovulation via modulation of angiotensin II receptors in in vitro ovarian follicle culture models. *J Tissue Eng Regen Med, 11*(11), 3100-3110. doi:10.1002/term.2214

Kim, Y. J., Ku, S. Y., Jee, B. C., Suh, C. S., Kim, S. H., Choi, Y. M., . . . Moon, S. Y. (2010). A comparative study on the outcomes of in vitro fertilization between women with polycystic ovary syndrome and those with sonographic polycystic ovary-only in GnRH antagonist cycles. *Arch Gynecol Obstet, 282*(2), 199-205. doi:10.1007/s00404-010-1401-9

Kim, Y. J., Ku, S. Y., Kim, Y. Y., Liu, H. C., Chi, S. W., Kim, S. H., . . . Moon, S. Y. (2013). MicroRNAs transfected into granulosa cells may regulate oocyte meiotic competence during in vitro maturation of mouse follicles. *Hum Reprod, 28*(11), 3050-3061. doi:10.1093/humrep/det338

Kim, Y. J., Ku, S. Y., Rosenwaks, Z., Liu, H. C., Chi, S. W., Kang, J. S., . . . Moon, S. Y. (2010). MicroRNA expression profiles are altered by gonadotropins and vitamin C status during in vitro follicular growth. *Reprod Sci, 17*(12), 1081-1089. doi:10.1177/1933719110377663

Kim, Y. J., Park, K. E., Kim, Y. Y., Kim, H., Ku, S. Y., Suh, C. S., . . . Choi, Y. M. (2017). Effects of Estradiol on the Paracrine Regulator Expression of In Vitro Maturated Murine Ovarian Follicles. *Tissue Engineering and Regenerative Medicine, 14*(1), 31-38. doi:10.1007/s13770-016-0006-1

Kim, Y. J., Shin, J. H., Hur, J. Y., Kim, H., Ku, S. Y., & Suh, C. S. (2017). Predictive value of serum progesterone level on beta-hCG check day in women with previous repeated miscarriages after in vitro fertilization. *PLoS One, 12*(7), e0181229. doi:10.1371/journal.pone.0181229

Kim, Y. Y., Kang, B. C., Yun, J. W., Ahn, J. H., Kim, Y. J., Kim, H., . . . Ku, S. Y. (2019). Expression of Transcripts in Marmoset Oocytes Retrieved during Follicle

Isolation Without Gonadotropin Induction. *Int J Mol Sci, 20*(5). doi:10.3390/ijms20051133

Kim, Y. Y., Kim, H., Ku, S. Y., Suh, C. S., Kim, S. H., Choi, Y. M., & Kim, S. J. (2016). Effects of Estrogen on Intracellular Calcium-Related T-Lymphocyte Function. *Tissue Engineering and Regenerative Medicine, 13*(3), 270-273. doi:10.1007/s13770-015-9070-1

Kim, Y. Y., Kim, Y. J., Cho, K. M., Kim, S. H., Park, K. E., Kang, B. C., . . . Ku, S. Y. (2016). The expression profile of angiotensin system on thawed murine ovaries. *Tissue Engineering and Regenerative Medicine, 13*(6), 724-731. doi:10.1007/s13770-016-0009-y

Kim, Y. Y., Ku, S. Y., Liu, H. C., Cho, H. J., Oh, S. K., Moon, S. Y., & Choi, Y. M. (2011). Cryopreservation of human embryonic stem cells derived-cardiomyocytes induced by BMP2 in serum-free condition. *Reprod Sci, 18*(3), 252-260. doi:10.1177/1933719110385130

Kim, Y. Y., Min, H., Kim, H., Choi, Y. M., Liu, H. C., & Ku, S. Y. (2017). Differential MicroRNA Expression Profile of Human Embryonic Stem Cell-Derived Cardiac Lineage Cells. *Tissue Engineering and Regenerative Medicine, 14*(2), 163-169. doi:10.1007/s13770-017-0051-4

Kim, Y. Y., Tamadon, A., & Ku, S. Y. (2017). Potential Use of Antiapoptotic Proteins and Noncoding RNAs for Efficient In Vitro Follicular Maturation and Ovarian Bioengineering. *Tissue Eng Part B Rev, 23*(2), 142-158. doi:10.1089/ten.TEB.2016.0156

Kim, Y. Y., Yun, J. W., Kim, J. M., Park, C. G., Rosenwaks, Z., Liu, H. C., . . . Ku, S. Y. (2016). Gonadotropin ratio affects the in vitro growth of rhesus ovarian preantral follicles. *J Investig Med, 64*(4), 888-893. doi:10.1136/jim-2015-000001

Kizuka-Shibuya, F., Tokuda, N., Takagi, K., Adachi, Y., Lee, L., Tamura, I., . . . Sugino, N. (2014). Locally existing endothelial cells and pericytes in ovarian stroma, but not bone marrow-derived vascular progenitor cells, play a central role in neovascularization during follicular development in mice. *J Ovarian Res, 7*, 10. doi:10.1186/1757-2215-7-10

Kniazeva, E., Hardy, A. N., Boukaidi, S. A., Woodruff, T. K., Jeruss, J. S., & Shea, L. D. (2015). Primordial Follicle Transplantation within Designer Biomaterial Grafts Produce Live Births in a Mouse Infertility Model. *Sci Rep, 5*, 17709. doi:10.1038/srep17709

Ko, I. K., Ju, Y. M., Chen, T., Atala, A., Yoo, J. J., & Lee, S. J. (2012). Combined systemic and local delivery of stem cell inducing/recruiting factors for in situ tissue regeneration. *Faseb j, 26*(1), 158-168. doi:10.1096/fj.11-182998

Kolli, S., Ahmad, S., Lako, M., & Figueiredo, F. (2010). Successful clinical implementation of corneal epithelial stem cell therapy for treatment of unilateral limbal stem cell deficiency. *Stem Cells, 28*(3), 597-610. doi:10.1002/stem.276

Kolli, S., Ahmad, S., Mudhar, H. S., Meeny, A., Lako, M., & Figueiredo, F. C. (2014). Successful application of ex vivo expanded human autologous oral mucosal epithelium for the treatment of total bilateral limbal stem cell deficiency. *Stem Cells, 32*(8), 2135-2146. doi:10.1002/stem.1694

Kossowska-Tomaszczuk, K., Pelczar, P., Guven, S., Kowalski, J., Volpi, E., De Geyter, C., & Scherberich, A. (2010). A novel three-dimensional culture system allows prolonged culture of functional human granulosa cells and mimics the ovarian environment. *Tissue Eng Part A, 16*(6), 2063-2073. doi:10.1089/ten.TEA.2009.0684

Ku, S. Y., Choi, Y. M., Suh, C. S., Kim, S. H., Kim, J. G., Moon, S. Y., & Lee, J. Y. (2002). Effect of gonadotropins on human endometrial stromal cell proliferation in vitro. *Arch Gynecol Obstet, 266*(4), 223-228. doi:10.1007/s00404-002-0292-9

L'Heureux , N., McAllister , T. N., & de la Fuente , L. M. (2007). Tissue-Engineered Blood Vessel for Adult Arterial Revascularization. *New England Journal of Medicine, 357*(14), 1451-1453. doi:10.1056/NEJMc071536

Laronda, M. M., Jakus, A. E., Whelan, K. A., Wertheim, J. A., Shah, R. N., & Woodruff, T. K. (2015). Initiation of puberty in mice following decellularized ovary transplant. *Biomaterials, 50*, 20-29. doi:10.1016/j.biomaterials.2015.01.051

Laronda, M. M., Rutz, A. L., Xiao, S., Whelan, K. A., Duncan, F. E., Roth, E. W., . . . Shah, R. N. (2017). A bioprosthetic ovary created using 3D printed microporous scaffolds restores ovarian function in sterilized mice. *Nat Commun, 8*, 15261. doi:10.1038/ncomms15261

Lass, A., Silye, R., Abrams, D. C., Krausz, T., Hovatta, O., Margara, R., & Winston, R. M. (1997). Follicular density in ovarian biopsy of infertile women: a novel method to assess ovarian reserve. *Human Reproduction, 12*(5), 1028-1031.

Lauer, G., & Schimming, R. (2001). Tissue-engineered mucosa graft for reconstruction of the intraoral lining after freeing of the tongue: a clinical and immunohistologic study. *J Oral Maxillofac Surg, 59*(2), 169-175; discussion 175-167. doi:10.1053/joms.2001.20489

Lawson, J. H., Glickman, M. H., Ilzecki, M., Jakimowicz, T., Jaroszynski, A., Peden, E. K., . . . Niklason, L. E. (2016). Bioengineered human acellular vessels for dialysis access in patients with end-stage renal disease: two phase 2 single-arm trials. *The Lancet, 387*(10032), 2026-2034. doi:https://doi.org/10.1016/S0140-6736(16)00557-2

Lee, S. H., Lee, S., Jun, H. S., Jeong, H. J., Cha, W. T., Cho, Y. S., . . . Cha, K. Y. (2003). Expression of the mitochondrial ATPase6 gene and Tfam in Down syndrome. *Mol Cells, 15*(2), 181-185.

Liu, D., & Pan, F. (2016). Advances in cryopreservation of organs. *J Huazhong Univ Sci Technolog Med Sci, 36*(2), 153-161. doi:10.1007/s11596-016-1559-x

Luyckx, V., Dolmans, M. M., Vanacker, J., Legat, C., Fortuno Moya, C., Donnez, J., & Amorim, C. A. (2014). A new step toward the artificial ovary: survival and proliferation of isolated murine follicles after autologous transplantation in a fibrin scaffold. *Fertil Steril, 101*(4), 1149-1156. doi:10.1016/j.fertnstert.2013.12.025

Luyckx, V., Dolmans, M. M., Vanacker, J., Scalercio, S. R., Donnez, J., & Amorim, C. A. (2013). First step in developing a 3D biodegradable fibrin scaffold for an artificial ovary. *J Ovarian Res, 6*(1), 83. doi:10.1186/1757-2215-6-83

Ma, H., Marti-Gutierrez, N., Park, S. W., Wu, J., Lee, Y., Suzuki, K., . . . Mitalipov, S. (2017). Correction of a pathogenic gene mutation in human embryos. *Nature, 548*(7668), 413-419. doi:10.1038/nature23305

Macchiarini, P., Jungebluth, P., Go, T., Asnaghi, M. A., Rees, L. E., Cogan, T. A., . . . Birchall, M. A. Clinical transplantation of a tissue-engineered airway. *The Lancet, 372*(9655), 2023-2030. doi:10.1016/S0140-6736(08)61598-6

Magoffin, D. A. (2005). Ovarian theca cell. *Int J Biochem Cell Biol, 37*(7), 1344-1349. doi:10.1016/j.biocel.2005.01.016

Mandai, M., Watanabe, A., Kurimoto, Y., Hirami, Y., Morinaga, C., Daimon, T., . . . Takahashi, M. (2017). Autologous Induced Stem-Cell-Derived Retinal Cells for

Macular Degeneration. *N Engl J Med, 376*(11), 1038-1046. doi:10.1056/NEJMoa1608368
Manley, M. (2014). Near-infrared spectroscopy and hyperspectral imaging: non-destructive analysis of biological materials. *Chem Soc Rev, 43*(24), 8200-8214. doi:10.1039/c4cs00062e
Martinez-Madrid, B., Dolmans, M. M., Van Langendonckt, A., Defrere, S., & Donnez, J. (2004). Freeze-thawing intact human ovary with its vascular pedicle with a passive cooling device. *Fertil Steril, 82*(5), 1390-1394. doi:10.1016/j.fertnstert.2004.06.036
McAllister, T. N., Maruszewski, M., Garrido, S. A., Wystrychowski, W., Dusserre, N., Marini, A., . . . L'Heureux, N. Effectiveness of haemodialysis access with an autologous tissue-engineered vascular graft: a multicentre cohort study. *The Lancet, 373*(9673), 1440-1446. doi:10.1016/S0140-6736(09)60248-8
Meirow, D., Levron, J., Eldar-Geva, T., Hardan, I., Fridman, E., Zalel, Y., . . . Dor, J. (2005). Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *N Engl J Med, 353*(3), 318-321. doi:10.1056/NEJMc055237
Mhatre, P., & Mhatre, J. (2006). Orthotopic ovarian transplant--review and three surgical techniques. *Pediatr Transplant, 10*(7), 782-787. doi:10.1111/j.1399-3046.2006.00547.x
Mhatre, P., Mhatre, J., Modi, B., & Doshi, S. (2003). *The Orthotopic Ovarian Transplant in Turner's Syndrome* (Vol. 18).
Muhart, M., McFalls, S., Kirsner, R., Kerdel, F., & Eaglstein, W. H. (1997). Bioengineered skin. *Lancet, 350*(9085), 1142. doi:10.1016/s0140-6736(05)63788-9
Mumme, M., Barbero, A., Miot, S., Wixmerten, A., Feliciano, S., Wolf, F., . . . Jakob, M. Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial. *The Lancet, 388*(10055), 1985-1994. doi:10.1016/S0140-6736(16)31658-0
Myers, M., Britt, K. L., Wreford, N. G., Ebling, F. J., & Kerr, J. B. (2004). Methods for quantifying follicular numbers within the mouse ovary. *Reproduction, 127*(5), 569-580. doi:10.1530/rep.1.00095
Nisolle, M., Casanas-Roux, F., Qu, J., Motta, P., & Donnez, J. (2000). Histologic and ultrastructural evaluation of fresh and frozen-thawed human ovarian xenografts in nude mice. *Fertil Steril, 74*(1), 122-129.
Oktay, K., Buyuk, E., Veeck, L., Zaninovic, N., Xu, K., Takeuchi, T., . . . Rosenwaks, Z. (2004). Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. *Lancet, 363*(9412), 837-840. doi:10.1016/s0140-6736(04)15728-0
Olausson, M., Patil, P. B., Kuna, V. K., Chougule, P., Hernandez, N., Methe, K., . . . Sumitran-Holgersson, S. Transplantation of an allogeneic vein bioengineered with autologous stem cells: a proof-of-concept study. *The Lancet, 380*(9838), 230-237. doi:10.1016/S0140-6736(12)60633-3
Pang, Y., Tsigkou, O., Spencer, J. A., Lin, C. P., Neville, C., & Grottkau, B. (2015). Analyzing Structure and Function of Vascularization in Engineered Bone Tissue by Video-Rate Intravital Microscopy and 3D Image Processing. *Tissue Eng Part C Methods, 21*(10), 1025-1031. doi:10.1089/ten.TEC.2015.0091
Paulini, F., Vilela, J. M., Chiti, M. C., Donnez, J., Jadoul, P., Dolmans, M. M., & Amorim, C. A. (2016). Survival and growth of human preantral follicles after cryopreservation

of ovarian tissue, follicle isolation and short-term xenografting. *Reprod Biomed Online, 33*(3), 425-432. doi:10.1016/j.rbmo.2016.05.003

Pellegrini, G., Traverso, C. E., Franzi, A. T., Zingirian, M., Cancedda, R., & De Luca, M. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. *The Lancet, 349*(9057), 990-993. doi:10.1016/S0140-6736(96)11188-0

Pereira, N., Kelly, A. G., Stone, L. D., Witzke, J. D., Lekovich, J. P., Elias, R. T., . . . Rosenwaks, Z. (2017). Gonadotropin-releasing hormone agonist trigger increases the number of oocytes and embryos available for cryopreservation in cancer patients undergoing ovarian stimulation for fertility preservation. *Fertil Steril, 108*(3), 532-538. doi:10.1016/j.fertnstert.2017.06.027

Povoa, A., Xavier, P., Calejo, L., Soares, S., Sousa, M., Silva, J., . . . Beires, J. (2016). First transplantation of cryopreserved ovarian tissue in Portugal, stored for 10 years: an unexpected indication. *Reprod Biomed Online, 32*(3), 334-336. doi:10.1016/j.rbmo.2015.12.002

Prabhasawat, P., Ekpo, P., Uiprasertkul, M., Chotikavanich, S., Tesavibul, N., Pornpanich, K., & Luemsamran, P. (2016). Long-term result of autologous cultivated oral mucosal epithelial transplantation for severe ocular surface disease. *Cell Tissue Bank, 17*(3), 491-503. doi:10.1007/s10561-016-9575-4

Pradel, W., & Lauer, G. (2012). Tissue-engineered bone grafts for osteoplasty in patients with cleft alveolus. *Ann Anat, 194*(6), 545-548. doi:10.1016/j.aanat.2012.06.002

Premachandra, D. J., Woodward, B. M., Milton, C. M., Sergeant, R. J., & Fabre, J. W. (1990). Treatment of postoperative otorrhoea by grafting of mastoid cavities with cultured autologous epidermal cells. *Lancet, 335*(8686), 365-367.

Prigione, A., Lichtner, B., Kuhl, H., Struys, E. A., Wamelink, M., Lehrach, H., . . . Adjaye, J. (2011). Human induced pluripotent stem cells harbor homoplasmic and heteroplasmic mitochondrial DNA mutations while maintaining human embryonic stem cell-like metabolic reprogramming. *Stem Cells, 29*(9), 1338-1348. doi:10.1002/stem.683

Quarto , R., Mastrogiacomo , M., Cancedda , R., Kutepov , S. M., Mukhachev , V., Lavroukov , A., . . . Marcacci , M. (2001). Repair of Large Bone Defects with the Use of Autologous Bone Marrow Stromal Cells. *New England Journal of Medicine, 344*(5), 385-386. doi:10.1056/nejm200102013440516

R. Daniel Beauchamp, B. M. E., Kenneth Mattox. (2016). *Sabiston textbook of surgery : the biological basis of modern surgical practice* (C. Townsend Ed. Twentieth Edition ed.): Elsevier.

Rajabzadeh, A. R., Eimani, H., Mohseni Koochesfahani, H., Shahvardi, A. H., & Fathi, R. (2015). Morphological study of isolated ovarian preantral follicles using fibrin gel plus platelet lysate after subcutaneous transplantation. *Cell J, 17*(1), 145-152.

Rama , P., Matuska , S., Paganoni , G., Spinelli , A., De Luca , M., & Pellegrini , G. (2010). Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration. *New England Journal of Medicine, 363*(2), 147-155. doi:10.1056/NEJMoa0905955

Ramirez, B. E., Sanchez, A., Herreras, J. M., Fernandez, I., Garcia-Sancho, J., Nieto-Miguel, T., & Calonge, M. (2015). Stem Cell Therapy for Corneal Epithelium Regeneration following Good Manufacturing and Clinical Procedures. *Biomed Res Int, 2015*, 408495. doi:10.1155/2015/408495

Raya-Rivera, A., Esquiliano, D. R., Yoo, J. J., Lopez-Bayghen, E., Soker, S., & Atala, A. Tissue-engineered autologous urethras for patients who need reconstruction: an

observational study. *The Lancet, 377*(9772), 1175-1182. doi:10.1016/S0140-6736(10)62354-9

Raya-Rivera, A., Esquiliano, D. R., Yoo, J. J., Lopez-Bayghen, E., Soker, S., & Atala, A. (2011). Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. *Lancet, 377*(9772), 1175-1182. doi:10.1016/S0140-6736(10)62354-9

Raya-Rivera, A. M., Esquiliano, D., Fierro-Pastrana, R., Lopez-Bayghen, E., Valencia, P., Ordorica-Flores, R., . . . Atala, A. (2014). Tissue-engineered autologous vaginal organs in patients: a pilot cohort study. *Lancet, 384*(9940), 329-336. doi:10.1016/S0140-6736(14)60542-0

Raya-Rivera, A. M., Esquiliano, D., Fierro-Pastrana, R., López-Bayghen, E., Valencia, P., Ordorica-Flores, R., . . . Atala, A. Tissue-engineered autologous vaginal organs in patients: a pilot cohort study. *The Lancet, 384*(9940), 329-336. doi:10.1016/S0140-6736(14)60542-0

Revel, A., Laufer, N., Ben Meir, A., Lebovich, M., & Mitrani, E. (2011). Micro-organ ovarian transplantation enables pregnancy: a case report. *Human Reproduction, 26*(5), 1097-1103. doi:10.1093/humrep/der063

Reynaud, K., Cortvrindt, R., Smitz, J., & Driancourt, M. A. (2000). Effects of Kit Ligand and anti-Kit antibody on growth of cultured mouse preantral follicles. *Mol Reprod Dev, 56*(4), 483-494. doi:10.1002/1098-2795(200008)56:4<483::aid-mrd6>3.0.co;2-o

Ricardo, J. R., Cristovam, P. C., Filho, P. A., Farias, C. C., de Araujo, A. L., Loureiro, R. R., . . . Gomes, J. A. (2013). Transplantation of conjunctival epithelial cells cultivated ex vivo in patients with total limbal stem cell deficiency. *Cornea, 32*(3), 221-228. doi:10.1097/ICO.0b013e31825034be

Romagnoli, G., De Luca, M., Faranda, F., Bandelloni, R., Franzi, A. T., Cataliotti, F., & Cancedda, R. (1990). Treatment of posterior hypospadias by the autologous graft of cultured urethral epithelium. *N Engl J Med, 323*(8), 527-530. doi:10.1056/nejm199008233230806

Ross, M. H. (2011). Histology : a text and atlas : with correlated cell and molecular biology. In W. Pawlina (Ed.), *Histology*: [Place of publication not identified] Wolters Kluwer/Lippincott Williams & Wilkins Health.

Sangwan, V. S., Vemuganti, G. K., Singh, S., & Balasubramanian, D. (2003). Successful reconstruction of damaged ocular outer surface in humans using limbal and conjuctival stem cell culture methods. *Biosci Rep, 23*(4), 169-174.

Schmidt, K. L., Andersen, C. Y., Loft, A., Byskov, A. G., Ernst, E., & Andersen, A. N. (2005). Follow-up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation. *Human Reproduction, 20*(12), 3539-3546. doi:10.1093/humrep/dei250

Scholz, S. L., Thomasen, H., Hestermann, K., Dekowski, D., Steuhl, K. P., & Meller, D. (2016). [Long-term results of autologous transplantation of limbal epithelium cultivated ex vivo for limbal stem cell deficiency]. *Ophthalmologe, 113*(4), 321-329. doi:10.1007/s00347-015-0110-y

Schroder, C. P., Timmer-Bosscha, H., Wijchman, J. G., de Leij, L. F., Hollema, H., Heineman, M. J., & de Vries, E. G. (2004). An in vitro model for purging of tumour cells from ovarian tissue. *Human Reproduction, 19*(5), 1069-1075. doi:10.1093/humrep/deh244

Schwartz, S. D., Regillo, C. D., Lam, B. L., Eliott, D., Rosenfeld, P. J., Gregori, N. Z., . . . Lanza, R. Human embryonic stem cell-derived retinal pigment epithelium in patients

with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. *The Lancet, 385*(9967), 509-516. doi:10.1016/S0140-6736(14)61376-3

Shin'oka , T., Imai , Y., & Ikada , Y. (2001). Transplantation of a Tissue-Engineered Pulmonary Artery. *New England Journal of Medicine, 344*(7), 532-533. doi:10.1056/nejm200102153440717

Shin'oka, T., Matsumura, G., Hibino, N., Naito, Y., Watanabe, M., Konuma, T., . . . Kurosawa, H. (2005). Midterm clinical result of tissue-engineered vascular autografts seeded with autologous bone marrow cells. *J Thorac Cardiovasc Surg, 129*(6), 1330-1338. doi:https://doi.org/10.1016/j.jtcvs.2004.12.047

Shiroma, M. E., Botelho, N. M., Damous, L. L., Baracat, E. C., & Soares-Jr, J. M. (2016). Melatonin influence in ovary transplantation: systematic review. *J Ovarian Res, 9*(1), 33. doi:10.1186/s13048-016-0245-8

Siegel, R. L., Miller, K. D., & Jemal, A. (2016). Cancer statistics, 2016. *CA: A Cancer Journal For Clinicians, 66*(1), 7-30. doi:10.3322/caac.21332

Silber, S. J. (2012). Ovary cryopreservation and transplantation for fertility preservation. *Mol Hum Reprod, 18*(2), 59-67. doi:10.1093/molehr/gar082

Silber, S. J., DeRosa, M., Pineda, J., Lenahan, K., Grenia, D., Gorman, K., & Gosden, R. G. (2008). A series of monozygotic twins discordant for ovarian failure: ovary transplantation (cortical versus microvascular) and cryopreservation. *Human Reproduction, 23*(7), 1531-1537. doi:10.1093/humrep/den032

Silber, S. J., & Gosden, R. G. (2007). Ovarian transplantation in a series of monozygotic twins discordant for ovarian failure. *N Engl J Med, 356*(13), 1382-1384. doi:10.1056/NEJMc066574

Silber, S. J., Lenahan, K. M., Levine, D. J., Pineda, J. A., Gorman, K. S., Friez, M. J., . . . Gosden, R. G. (2005). Ovarian transplantation between monozygotic twins discordant for premature ovarian failure. *N Engl J Med, 353*(1), 58-63. doi:10.1056/NEJMoa043157

Sittadjody, S., Saul, J. M., Joo, S., Yoo, J. J., Atala, A., & Opara, E. C. (2013). Engineered multilayer ovarian tissue that secretes sex steroids and peptide hormones in response to gonadotropins. *Biomaterials, 34*(10), 2412-2420. doi:10.1016/j.biomaterials.2012.11.059

Smith, R. M., Shikanov, A., Kniazeva, E., Ramadurai, D., Woodruff, T. K., & Shea, L. D. (2014). Fibrin-mediated delivery of an ovarian follicle pool in a mouse model of infertility. *Tissue Eng Part A, 20*(21-22), 3021-3030. doi:10.1089/ten.TEA.2013.0675

Soares, M., Sahrari, K., Chiti, M. C., Amorim, C. A., Ambroise, J., Donnez, J., & Dolmans, M. M. (2015). The best source of isolated stromal cells for the artificial ovary: medulla or cortex, cryopreserved or fresh? *Human Reproduction, 30*(7), 1589-1598. doi:10.1093/humrep/dev101

Soares, M., Saussoy, P., Sahrari, K., Amorim, C. A., Donnez, J., & Dolmans, M. M. (2015). Is transplantation of a few leukemic cells inside an artificial ovary able to induce leukemia in an experimental model? *J Assist Reprod Genet, 32*(4), 597-606. doi:10.1007/s10815-015-0438-x

Steinke, M., Dally, I., Friedel, G., Walles, H., & Walles, T. (2015). Host-integration of a tissue-engineered airway patch: two-year follow-up in a single patient. *Tissue Eng Part A, 21*(3-4), 573-579. doi:10.1089/ten.TEA.2014.0200

Tamadon, A., Park, K.-H., Kim, Y. Y., Kang, B.-C., & Ku, S.-Y. (2016). Efficient biomaterials for tissue engineering of female reproductive organs. *Tissue Engineering and Regenerative Medicine, 13*(5), 447-454. doi:10.1007/s13770-016-9107-0

Telfer, E., Torrance, C., & Gosden, R. G. (1990). Morphological study of cultured preantral ovarian follicles of mice after transplantation under the kidney capsule. *J Reprod Fertil, 89*(2), 565-571.

Tsubota, K., Satake, Y., Kaido, M., Shinozaki, N., Shimmura, S., Bissen-Miyajima, H., & Shimazaki, J. (1999). Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. *N Engl J Med, 340*(22), 1697-1703. doi:10.1056/nejm199906033402201

Vacanti , C. A., Bonassar , L. J., Vacanti , M. P., & Shufflebarger , J. (2001). Replacement of an Avulsed Phalanx with Tissue-Engineered Bone. *New England Journal of Medicine, 344*(20), 1511-1514. doi:10.1056/nejm200105173442004

Van Eyck, A. S., Jordan, B. F., Gallez, B., Heilier, J. F., Van Langendonckt, A., & Donnez, J. (2009). Electron paramagnetic resonance as a tool to evaluate human ovarian tissue reoxygenation after xenografting. *Fertil Steril, 92*(1), 374-381. doi:10.1016/j.fertnstert.2008.05.012

Vanacker, J., Dolmans, M. M., Luyckx, V., Donnez, J., & Amorim, C. A. (2014). First transplantation of isolated murine follicles in alginate. *Regen Med, 9*(5), 609-619. doi:10.2217/rme.14.33

Vanacker, J., Luyckx, V., Amorim, C., Dolmans, M. M., Van Langendonckt, A., Donnez, J., & Camboni, A. (2013). Should we isolate human preantral follicles before or after cryopreservation of ovarian tissue? *Fertil Steril, 99*(5), 1363-1368.e1362. doi:10.1016/j.fertnstert.2012.12.016

Vanacker, J., Luyckx, V., Dolmans, M. M., Des Rieux, A., Jaeger, J., Van Langendonckt, A., . . . Amorim, C. A. (2012). Transplantation of an alginate-matrigel matrix containing isolated ovarian cells: first step in developing a biodegradable scaffold to transplant isolated preantral follicles and ovarian cells. *Biomaterials, 33*(26), 6079-6085. doi:10.1016/j.biomaterials.2012.05.015

Vassena, R., Heindryckx, B., Peco, R., Pennings, G., Raya, A., Sermon, K., & Veiga, A. (2016). Genome engineering through CRISPR/Cas9 technology in the human germline and pluripotent stem cells. *Hum Reprod Update, 22*(4), 411-419. doi:10.1093/humupd/dmw005

Walles, T., Biancosino, C., Zardo, P., Macchiarini, P., Gottlieb, J., & Mertsching, H. (2005). Tissue remodeling in a bioartificial fibromuscular patch following transplantation in a human. *Transplantation, 80*(2), 284-285.

Warnke, P. H., Springer, I. N. G., Wiltfang, J., Acil, Y., Eufinger, H., Wehmöller, M., . . . Terheyden, H. (2004). Growth and transplantation of a custom vascularised bone graft in a man. *The Lancet, 364*(9436), 766-770. doi:https://doi.org/10.1016/S0140-6736(04)16935-3

Watson, C. S., Alyea, R. A., Cunningham, K. A., & Jeng, Y. J. (2010). Estrogens of multiple classes and their role in mental health disease mechanisms. *Int J Womens Health, 2*, 153-166.

Wenners, A., Grambach, J., Koss, J., Maass, N., Jonat, W., Schmutzler, A., & Mundhenke, C. (2017). Reduced ovarian reserve in young early breast cancer patients: preliminary data from a prospective cohort trial. *BMC Cancer, 17*(1), 632. doi:10.1186/s12885-017-3593-x

White, Y. A., Woods, D. C., Takai, Y., Ishihara, O., Seki, H., & Tilly, J. L. (2012). Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. *Nat Med, 18*(3), 413-421. doi:10.1038/nm.2669

Wystrychowski, W., McAllister, T. N., Zagalski, K., Dusserre, N., Cierpka, L., & L'Heureux, N. (2014). First human use of an allogeneic tissue-engineered vascular graft for hemodialysis access. *J Vasc Surg, 60*(5), 1353-1357. doi:10.1016/j.jvs.2013.08.018

Zakaria, N., Possemiers, T., Dhubhghaill, S. N., Leysen, I., Rozema, J., Koppen, C., . . . Tassignon, M. J. (2014). Results of a phase I/II clinical trial: standardized, non-xenogenic, cultivated limbal stem cell transplantation. *J Transl Med, 12*, 58. doi:10.1186/1479-5876-12-58

Table 1. Clinical examples in tissue and organ engineering

Organ	#	Condition	Transplantation	Implant Composition	Clinical Trials Phase	Reference
Bladder	1	Myelomenigocele, candidates for cytoplasty	Autologous engineered bladder construct	Cultured urothelial, muscle cells from a bladder biopsy, seeded on a biodegradable (collagen, polyglycolic acid) bladder-shaped scaffold	Phase II	(Atala, Bauer, Soker, Yoo, & Retik)
Bone	1	Long bone nonunion in congential psuedarthrosis or carcinologic resection, bone tumor, and bone pseudarthrosis	Autologous scaffold-free osteogenic 3D graft	Autologous adipose-derived stem cells in an allogeneic demineralized bone matrix	Phase I/II	(Dufrane et al., 2015)
	2	Partial avulsion of a thumb	Tissue engineered distal phalanx	Autologous periosteum cells encapsulated in a calcium alginate hydrogel saturated in a natural coral (porous hydroxyapatite) implant		(Vacanti , Bonassar , Vacanti , & Shufflebarger 2001)
	3	Bone deficiency of a maxillary sinus	Autologous stem cell delivery in a scaffold	Autologous bone marrow derived stem cells delivered onto a β-tricalcium phosphate scaffold		(Kaigler et al., 2015)
	4	A bone cyst	Tissue engineered bone	Bone marrow mesenchymal stem cells in an allogeneic demineralized bone matrix		(Hou et al., 2016)
	5	A large bone defect	Tissue engineered bone	Bone marrow derived osteoprogenitor cells expanded *ex vivo* and placed on a macroporous hydroxyapatite scaffold		(Quarto et al., 2001)
	6	A mandibular discontinuity defect	Engineered bone	A titanium mesh cage filled with bone mineral blocks (bone marrow biopsy specimens of undifferentiated cells) and recombinant human BMP7		(Warnke et al., 2004)
	7	Cleft alveolus	Tissue engineered bone	Cells from a bone biopsy seeded in a collagen matrix		(Pradel & Lauer, 2012)
Cartilage	1	Full-thickness cartilage defects in	Tissue engineered cartilage like tissue	Autologous chondrocytes in a atelo-collagen gel or cultured on a collagen membrane	Up to phase III	(Adachi et al., 2014; Mumme et al.)

		knee, articular cartilage lesions				
	2	Nasal cartilage defects due to tumor resection	Engineered autologous cartilage tissue	Chondrocytes from a nasal septum biopsy cultured with autologous serum onto collagen type I and III membranes		(Fulco et al.)
Cornea	1	Limbal stem cell deficiency (LSCD), damaged ocular surface	*Ex vivo* expanded limbal epithelium	Autologous and allogeneic limbal epithelial stem cells cultivated on an amniotic membrane	Up to Phase IV	(Kolli, Ahmad, Lako, & Figueiredo, 2010; Ramirez et al., 2015; Ricardo et al., 2013; Sangwan, Vemuganti, Singh, & Balasubramanian, 2003; Scholz et al., 2016; Tsubota et al., 1999; Zakaria et al., 2014)
	2	Limbal stem cell deficiency (LSCD), ocular surface burns	*Ex vivo* expanded autologous limbal epithelium	Autologous limbal stem cells cultivated in a fibrin matrix		(Guo, Pi, Dong, & Zhu, 2013; Rama et al., 2010)
	3	Limbal stem cell deficiency (LSCD)	Autologous cultured corneal (LSC) epithelium	Autologous superior forniceal conjunctiva (bilateral LSCD cases) or superior limbal epithelium (unilateral LSCD case) ex-vivo expanded on contact lens; autologous limbal or conjunctival epithelium harvested on the concave surface of silicone hydrogel contact lenses, cultured in autologous serum		(Bobba, Chow, Watson, & Di Girolamo, 2015; Di Girolamo et al., 2009; Pellegrini et al.)
	4	Limbal stem cell deficiency (LSCD)	*Ex vivo* expanded autologous oral mucosal epithelium	*Ex vivo* expanded autologous oral mucosal epithelium on an human amniotic membrane		(Burillon et al., 2012; Chen et al., 2009; Dobrowolski et al., 2015; Kolli et al., 2014; Prabhasawat et al., 2016)
Esophagus	1	A direct communication between hypopharynx and mediastinum (from a car accident, mediastinal abscess)	*In vivo* esophageal regeneration	A stent covered with ECM and autologous platelet-rich plasma adhesive gel	N/A (Case report)	(Dua, Hogan, Aadam, & Gasparri)
Gingiva/	1	A mucogingival defect or a lack of keratinized	Tissue-engineered human *ex vivo*-	Autologous oral keratinocytes expanded on an acellular dermal matrix	Phase II	(Izumi, Neiva, &

Mouth		gingiva on a non-molar tooth	produced oral mucosa equivalent (EVPOME)			Feinberg, 2013)
	2	Large intraoral wounds (due to oral squamous cell carcinoma)	Tissue engineered mucosa	Cultured keratinocytes from a hard palate biopsy specimen (using vaseline gauze as a carrier)		(Lauer & Schimming, 2001)
Retina	1	Age-related macular degeneration, Stargardt's macular dystrophy	Engineered retinal pigment epithelium	Human embryonic stem cells	Phase I/II	(Schwartz et al.)
	2	Aged macular degeneration	Engineered retinal pigment epithelium	Induced pluripotent stem cells (iPSCs) derived retinal pigment epithelial cell		(Mandai et al., 2017)
Skin	1	Burn wounds	Temporary biosynthetic skin	Human neonatal fibroblasts cultured on a synthetic dressing (a nylon mesh fabric) covered with a thin layer of silicone rubber membrane;	Up to Phase III	(Hansbrough et al., 1997)
	2	Burns, acute wounds, venous ulcers	Bioengineered skin	Epidermis and dermis made of type I bovine collagen and cultured allogeneic cells (keratinocytes and fibroblasts) isolated from human neonatal foreskin		(Muhart, McFalls, Kirsner, Kerdel, & Eaglstein, 1997)
	3	Postoperative otorrhea, large burn wounds	Cultured autologous epidermal cells	Autologous keratinocytes and skin specimen on a gauze		(De Luca et al., 1989; Gallico, O'Connor, Compton, Kehinde, & Green, 1984; Premachandra, Woodward, Milton, Sergeant, & Fabre, 1990)
	4	Pediatric burns	Tissue engineered fetal skin construct	Fetal skin from a donor		(Hohlfeld et al., 2005)
Trachea	1	Long segment congenital tracheal stenosis and pulmonary sling, end staged left main bronchus with malacia	Tissue engineered trachea	Bone marrow mesenchymal stem cells seeded onto a scaffold with patches of autologous epithelium	Phase I (suspended)	(Martin J. Elliott et al.; Gonfiotti et al.; Hamilton et al., 2015; Macchiarini et al.)
	2	A primary cancer of distal trachea and main bronchi	Stem-cell-seeded bio-artificial nanocomposite as a functional trachea	A tailored bio-artificial nanocomposite seeded with autologous bone-marrow mononuclear cells		(Jungebluth et al.)

	3	A post-traumatic tracheal defect	Tissue engineered airway patch	A porcine decellularized scaffold seeded with recipient's autologous microvascular endothelial cells and skeletal muscle cells		(Steinke, Dally, Friedel, Walles, & Walles, 2015)
	4	A tracheobronchial anastomosis defect	Bio-artificial fibromuscular tracheal patch	Autologous muscle cells and fibroblasts in collagen		(Walles et al., 2005)
Urethra	1	Urethral defects	Tissue engineered tubularized urethra	Muscle and epithelial cells (from bladder biopsy) expanded and seeded onto a tubularized polyglycolic acid:poly(lactide-co-glycolide acid) scaffold	Phase I	(A. Raya-Rivera et al.)
	2	Congenital posterior hypospadias	Biopsied urethral meatus epithelium cultured *in vitro*	Biopsied urethral meatus epithelium cultured *in vitro*		(Romagnoli et al., 1990)
	3	Urethral stricture	Neourethra (an engineered urethra)	A collagen based inert matrix from decellularized cadaveric human bladder tissue		(A. el-Kassaby, AbouShwareb, & Atala, 2008; A. W. El-Kassaby, Retik, Yoo, & Atala, 2003)
Urinary Conduit	1	Bladder cancer undergoing cystectomy	Tissue engineered neo-urinary conduit (NUC)	Autologous fat smooth muscle cells seeded onto a synthetic, biodegradable poly(lactic-co-glycolic acid) scaffold	Phase I	(T. Bivalacqua et al.; Trinity Bivalacqua et al.; Kates et al., 2015)
Vagina	1	Congenital vaginal aplasia by Mayer-Rokitansky-Küster-Hauser syndrome (MRKHs)	Tissue engineered autologous vaginal organ	Autologous epithelial and muscle cells from vulvar biopsy onto biodegradable scaffold	N/A (Cohort study)	(A. M. Raya-Rivera et al.)
Vessels	1	End stage renal disease	Bioengineered human acellular vessels for dialysis access	Human vascular smooth muscle cells from donors cultured on a biodegradable polymer	Up to Phase II	(Lawson et al., 2016)
	2	End stage renal disease	Tissue engineered vascular graft	Sheets of autologous or allogeneic fibroblasts wrapped around a stainless steel mandrel with/without autologous endothelial cell seeding in the lumen		(McAllister et al.; Wystrychowski et al., 2014)
	3	End stage renal disease	Tissue engineered blood vessel	Sheet-based engineering of autologous fibroblasts and endothelial cells harvested from skin and superficial vein biopsy		(L'Heureux , McAllister , & de la Fuente 2007)
	4	Single ventricle physiology,	Tissue engineered vascular graft	Autologous bone marrow derived mononuclear cells on a biodegradable scaffold		(Hibino et al., 2010;

	congenital heart anomaly		(polyglycolic acid and ε-caprolactone/l-lactide)	Shin'oka et al., 2005)
5	Pediatric congenital heart anomaly	Tissue engineered pulmonary artery	Cells from peripheral vein expanded and seeded on a scaffold (polycaprolactone-polylactic acid copolymer and woven polyglycolic acid)	(Shin'oka , Imai , & Ikada 2001)
6	Extrahepatic portal vein obstruction	Bioengineered allogeneic vein	A decellularized allogeneic donor iliac vein recellurlarized with endothelial and smooth muscle cells derived from autologous bone marrow stem cells	(Olausson et al.)

Table 2. Various follicle delivery materials in animal transplantation studies of the artificial ovary [modified from (Amorim & Shikanov, 2016)] H: human, M: mice

Year	Delivery Material	Species	Follicle	Grafting Location	Grafting Period	Results	Reference
1990	Collagen	M-M	Pre-antral follicles	Kidney capsule	2-12 days	Mature oocytes, offspring through IVF	(Telfer et al., 1990)
1990	Plasma clot	M-M	Primordial follicles	Ovarian bursa	4-12 weeks	Offspring through natural mating	(Gosden, 1990)
1993	Plasma clot	M-M	Primordial follicles from cryopreserved ovarian tissue	Ovarian bursa	6-12 weeks	Offspring through natural mating	(Carroll & Gosden, 1993)
2008	Fibrin	M-M	Pre-antral follicles	Peritoneal pocket	1 week	Antral follicles	(Dolmans et al., 2008)
2008	Plasma clot	H-M	Pre-antral follicles	Ovarian bursa	5 months	Antral follicles	(Dolmans et al., 2008)
2012	Alginate-matrigel	M-M	Pre-antral follicles	Peritoneal pocket	1 week	Antral follicles	(Vanacker et al., 2012)
2014	Alginate	M-M	Pre-antral follicles	Peritoneal pocket	1 week	Antral follicles	(Vanacker et al., 2014)
2014	Fibrin	M-M	Pre-antral follicles	Ovarian bursa	3 weeks	Corpora lutea, antral follicles	(Smith et al., 2014)
2015	Fibrin-VEGF, fibrin-alginate, fibrin-collagen	M-M	Pre-antral follicles	Ovarian bursa	More than 9 days	Offspring in fibrin-VEGF	(Kniazeva et al., 2015)
2015	fibrin-platelet lysate	M-M	Pre-antral follicles	Subcutaneous pocket	2 weeks	Antral follicles	(Rajabzadeh et al., 2015)
2016	Fibrin, fibrin-hyaluronic acid	H-M	Pre-antral follicles	Peritoneal pocket	1 week	Follicle growth	(Paulini et al., 2016)
2016	Poly (ethylene glycol) vinyl-sulfone (PEG-VS)	M-M	Immature follicles	Ovarian bursa	60 days	Antral follicles and corpora lutea, decreased FSH level	(J. Kim et al., 2016)
2017	Gelatin, 3D printed	M-M	Primordial, primary, secondary follicles	Ovarian bursa	10 weeks	Offspring through natural mating	(Laronda et al., 2017)

Table 3. Different surgical procedures in ovarian transplantations or tissue engineered product transplantations in human

		Whole Ovary Transplantation	Ovarian Cortex Transplantation	Orthotopic Ovarian Tissue Transplantation	Tissue-engineered Trachea	Tissue-engineered Vagina
Pre-surgery	Preparation	Identification of a donor	Stopped hormone replacement therapy (HRT) for 2 weeks prior to surgery	Ovarian tissue cryopreservation and thawing for 2 min at room temperature and in another 2 min at 37 °C	Bone marrow biopsy, a sterile donor trachea transferred in PBS with penicillin, streptomycin, amphotericin B	Biopsied vulvar cells cultured for 3-5 weeks in 5% CO_2 at 37 °C
	Evaluations prior to surgery	Immunological match with donor by blood group, HLA, lymphocyte	Serum hormone level, HIV/hepatitis screening	Digital optical microscopy for follicles in tissue, serum hormone level	CT scan, donor trachea sterility and absence of HLA-I+ cells evaluation	Immunocytochemistry, sterility check, MTT assay of the scaffold
	Total time till surgery	Can be done immediately if a donor is identified	About two weeks after stopping HRT	Dependent on length of chemotherapy	About 3 days; surgery done soon after bone marrow biopsy and preparation of a donated trachea	5-6 weeks of preparation time
Surgery	Approach / Cavity location	Orthotopic position transperitonially	Minimal laparotomy through a 3.5 cm incision above the pubis	Furrow created near ovarian vessels and fimbria(J. Donnez et al., 2004) Laparoscopically transplanted into either broad ligament region or ovarian fossa (Povoa et al., 2016) Three pairs of 5-mm transverse incisions in the left ovary through the tunica albuginea(Meirow et al., 2005)	Sternotomy	Perineal approach, ventral rectal and posterior bladder wall cavity
	Transplanted organ	A fresh whole ovary detached from a donor	Ovary dissected *ex vivo*, cortical tissue trimmed to 1-2mm and medullary tissue removed	Small size strips or fragments of ovarian tissue	A sterile donor trachea filled with autologous bone marrow mesenchymal stem cells	Intestinal submucosa segments filled with cultured epithelial and muscle cells

	Implant size	A whole ovary	One third of the donor ovarian cortex about 1 mm thickness is implanted (left two thirds are cryopreserved for future usage)	A large strip and 30-35 small cubes after thawing or 10 x 4-5 mm 6 cortical pieces (J. Donnez et al., 2004) Four 2 x 2 cubes after thawing (Povoa et al., 2016) Three 1.5 x 0.5 x 0.1-0.2 cm strips in cortex cavity (Meirow et al., 2005) 300-350 μm thick micro-organ (MO) fragments of ovarian cortex tissue (Revel et al., 2011)	Customized for patient's anatomical need (trachea length and size)	Customized for patient's anatomical need (vagina length and size)
	Vascularization/ Anastomosis	Donor ovarian artery to recipient inferior epigastric artery, donor ovarian vein to recipient external iliac vein (P. Mhatre & Mhatre, 2006; Pravin Mhatre et al., 2003) Donor ovarian vein to recipient ovarian vein, donor ovarian artery to recipient ovarian artery with 9-0 or 10-0 nylon sutures (Silber et al., 2008)	Donor ovarian cortex was laid over the raw medulla of each recipient ovary and sutured on to the medulla. It is less invasive and reduces risk and recovery time than an entire ovary vascular transplantation.	7 days before implantation, an incision beneath the right ovarian hilus was made to create a peritoneal window and window edge was coagulated to induce angiogenesis (J. Donnez et al., 2004)	Superiorly and inferiorly anastomosed using horizontal mattress interrupted sutures Omentum wrapping to reduce the possibility of future fistulae and increase graft vascularity	Vascularized to vestigial tissues, hypoplastic uterus or hemi-uterus with absorbable suture
	Medication	None	None	None	Human recombinant erythropoietin (hrEPO), G-CSF, TGF-beta	None
Post-surgery	Medication	Immunosuppression with cyclosporine and prednisolone	None	None	hrEPO, G-CSF	None
	Mechanical Support	N/A	N/A	N/A	Stent insertion, bronchoscopy or balloon dilatation under fluoroscopy regularly for 6 month	Stent insertion for 8 weeks

	Evaluation/ Follow-ups	Menstruation history, MRI after 14 months, CD4 / WBC count, USG, and Doppler for allograft rejection monitoring	Serum hormone level, menstruation history	Serum hormone Level, USG, MRI, vaginal echography	Endoscopy, cytological evaluation, CT	Vaginoscopy, biopsy at 3, 6, 12 months and yearly afterwards, self-questionnaire on sexual function, MRI
	Normalization Period	First menstruation after 100 days	First menstruation after 65-93 days	Approximately 5 months	6 months	7 days of hospitalization, finally no stent insertion 2 months after surgery
Reference		(P. Mhatre & Mhatre, 2006; Pravin Mhatre et al., 2003)	(Silber et al., 2008; Silber & Gosden, 2007; Silber et al., 2005)	(J. Donnez et al., 2004; J. Donnez, Dolmans, et al., 2006; J. Donnez, Martinez-Madrid, et al., 2006; Meirow et al., 2005; Povoa et al., 2016; Revel et al., 2011)	(Martin J. Elliott et al.)	(A. M. Raya-Rivera et al.)

Table 4. Potential evaluation methods of the artificial ovary in humans

	Evaluated Subjects	Methods	Reference
Before Transplantation	Ovarian reserve and function	Serum hormone (AMH, LH, FSH, Estradiol) level, ultrasonography (USG)	(Belaisch-Allart et al., 1991; Lass et al., 1997; Schmidt et al., 2005; Wenners et al., 2017)
	Cryopreservation efficiency	Florescent staining for viability and morphological evaluation under microscope	(J. Donnez et al., 2004; Martinez-Madrid et al., 2004)
After Transplantation	Oxygenation	Electron paramagnetic resonance, PET/CT, NIRS, HSI usage, intravital microscopy	(Dejust et al., 2017; Fisher et al., 2016; Holmer et al., 2016; Jarraya et al., 2016; Manley, 2014; Pang et al., 2015)
	Function	Follicle size and count via USG, serum hormone (AMH, LH, FSH, Estradiol) level, physical examination including menstruation period and pregnancy Biopsy sample follicle count/histological evaluation under microscopy or TEM, TUNEL assay, immunohistochemistry and western blot analysis (e.g. Ki-67, Inhibin-α, 3β-HSD, Laminin, Calretinin, P450 aromatase, FSHR antibody, HMGB1)	(J. Donnez et al., 2004; J. Donnez, Martinez-Madrid, et al., 2006; Meirow et al., 2005; P. Mhatre & Mhatre, 2006; Pravin Mhatre et al., 2003; Povoa et al., 2016; Silber et al., 2005)
	Immune response	CRP, CD4/WBC count, USG	(P. Mhatre & Mhatre, 2006; Pravin Mhatre et al., 2003)

Table 5. Advantages and disadvantages of different sources for gametes in the artificial ovary (Bhartiya et al., 2014; Hanna & Hennebold, 2014; R. Daniel Beauchamp, 2016)

Sources	Advantages	Disadvantages
Biopsied ovarian cells	- Autologous origin - Existing protocols to isolate follicles and oocytes	- May contain malignant cells
Induced pluripotent stem Cells (iPSCs)	- Autologous origin	- Conversion to gametes still in research - Safety issues due to possible mutagenesis and proto-oncogenic reprogramming factors
Embryonic stem cells (ESCs)	- Pluripotency - Unlimited ability for self-renewal	- Conversion to gametes still in research - Ethical issues - Biological parenthood not guaranteed - Immune mismatch - Tumorigenesis - Possible contamination (immunogenic nonhuman sialic acid and virus) from animal cell layers but a feeder free culture is also possible
Oogonial stem cells (OSCs), very small embryonic like (VSEL) stem cells	- Equivalent to primordial germ cells - Autologous origin - No need to cryopreserve gonadal tissue prior to oncotherapy because these cells survive oncotherapy	- Questioned existences and epigenetic normalities - Optimization and characterization of conditions for OSC culture and development still in research

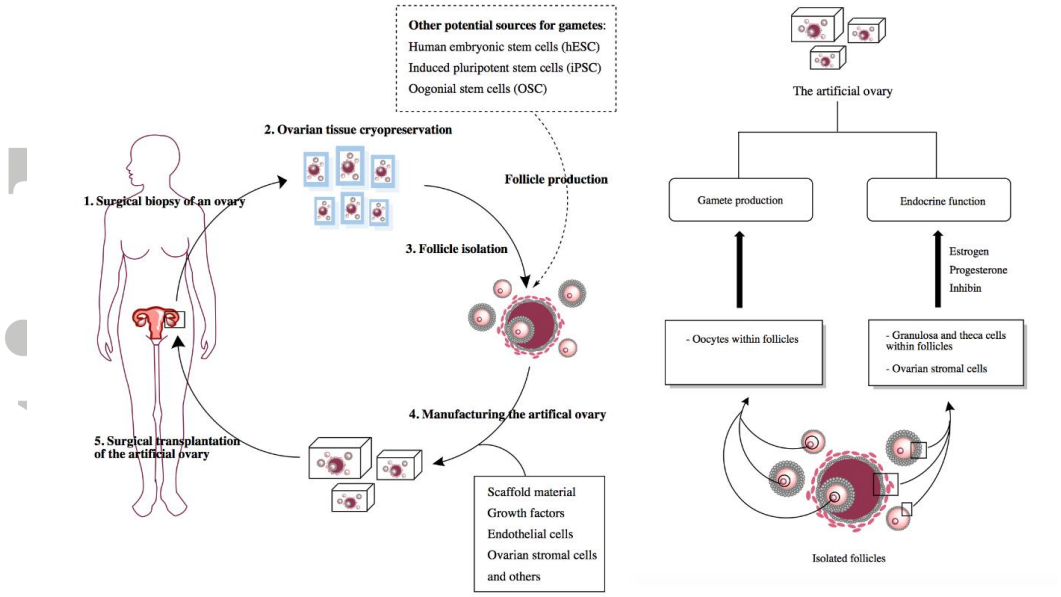

Figure 1. Manufacturing and transplanting of the artificial ovary

Theoretically, the artificial ovary aims to restore reproductive and/or endocrine function of ovaries. Ovarian tissue or entire ovaries are retrieved and cryopreserved. When a pregnancy is desired, follicles are isolated from thawed ovarian tissue and are placed in a scaffold equipped with or without growth factors, endothelial cells and ovarian stromal cells. When successfully transplanted, oocytes within follicles can provide female gametes for establishing a pregnancy whereas granulosa and theca cells of follicles along with ovarian stromal cells can produce female sex steroid hormones such as estrogen and progesterone.