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Mini-Review

Developments in reproductive biology and medicine

Human ovarian tissue xenotransplantation: advancements, challenges, and future perspectives

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ABSTRACT

Ovarian tissue cryopreservation and transplantation has emerged as a promising fertility preservation technique for individuals facing premature ovarian insufficiency due to various medical conditions or treatments. Xenotransplantation, involving the transplantation of ovarian tissue into animal hosts, has played a pivotal role in refining ovarian tissue cryopreservation and transplantation techniques and addressing key challenges. This review provides a comprehensive overview of the current landscape of ovarian tissue xenotransplantation research, focusing on its applications in investigating ovarian biology, optimizing ovarian tissue cryopreservation and transplantation protocols, and assessing safety concerns. It also explores the utilization of xenografting of human ovarian tissue in mouse models in the last 10 years. Key findings from preclinical studies investigating grafting site optimization, cryopreservation protocol refinement, the development of strategies to mitigate chemotherapy-induced damage, follicle development, tissue revascularization, and the risk of malignant cell reintroduction are summarized. Moreover, the review examines the ethical considerations surrounding the use of animals in ovarian tissue xenotransplantation research and suggests emerging alternative models that aim to minimize animal use while maximizing clinical relevance.

Keywords: ovary / cryopreservation / fertility / mouse / xenografting

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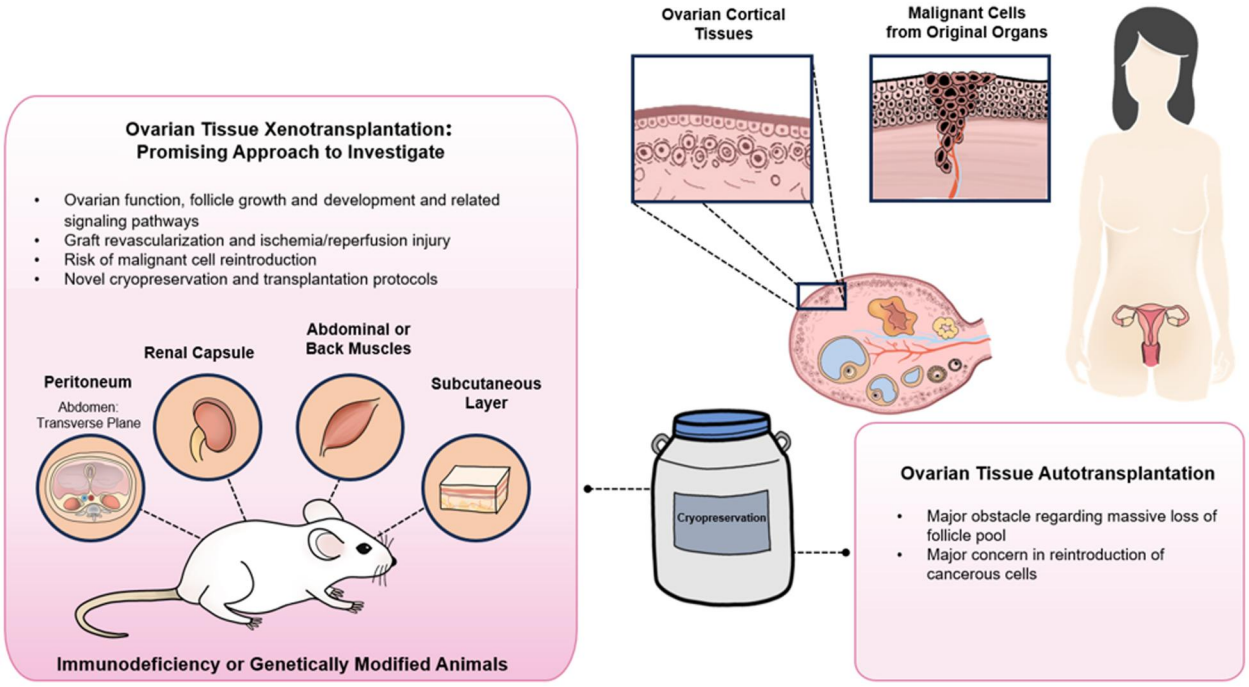
GRAPHICAL ABSTRACT



Modeling OTCT with OTX: enhancing understanding of OTCT. OTCT, ovarian tissue cryopreservation and autotransplantation; OTX, ovarian tissue xenotranspantation.

Introduction

To preserve female fertility and improve the quality of life for women experiencing premature menopause, various fertility preservation methods have been developed. Ovarian transplantation methods have been widely proposed as an alternative to restore ovarian function, complementing options like oocyte and embryo cryopreservation for fertility preservation. One of the main challenges is ensuring the safety and effectiveness of ovarian tissue cryopreservation (Roness and Meirow, 2019; Dolmans et al., 2021). However, since the beginning of the 21st century, ovarian tissue transplantation has effectively restored endocrine and reproductive capability in individuals with ovarian insufficiency (Oktay and Karlikaya, 2000; Donnez et al., 2004).

Ovarian tissue cryopreservation and autotransplantation (OTCT) has been presented as a viable option for fertility preservation; meanwhile, xenotransplantation has emerged as a critical strategy with a pivotal role in refining strategies for OTCT. More specifically, these methods aim to restore reproductive ability in patients subjected to gonadotoxic treatments and exposed to alkylating agents or radiotherapy that leads to premature ovarian insufficiency (Chow et al., 2016; Lambertini et al., 2018).

OTCT is particularly indicated for patients unable to undergo embryo or mature oocyte cryopreservation after ovarian stimulation, such as prepubertal girls or for those whose cancer treatment must be initiated promptly (Dolmans et al., 2021). To date, over 200 live births have been reported through the utilization of OTCT (>41% pregnancy rate, 21.6–30% live birth rate) (Khattak et al., 2022), and nearly all transplanted patients have experienced restoration of endocrine function (Diaz-Garcia et al., 2018). Despite promising results, standardized protocols for transport, cryopreservation, and transplantation remain unresolved; improved protocols would minimize follicle loss from ischemia, rapid follicle recruitment post-grafting, and possible malignant cells in ovarian tissues (Dolmans et al., 2021).

Comparatively, ovarian tissue xenotransplantation (OTX) consists of the implantation of tissue into a recipient of a different species to support follicular development of frozen–thawed ovarian samples. It has also delved into additional aspects that contribute to the advancement of techniques in this field in experimental trials over the past decades, specifically in research related to the preservation of ovarian tissue and the restoration of its functionality following thawing. Xenografting has emerged as a crucial model for studying ovary biology, including the effects of different drugs on the follicle population. Additionally, this approach provides a valuable platform to assess the potential re-establishment of diseases that may be present in the transplanted tissue (Shapira et al., 2018). Certain mouse strains serve as bioincubators with the capacity to support the growth of neoplastic cells (Nguyen et al., 2021). Using immunodeficient mice helps overcome some limitations of traditional transplantation methods by avoiding immune rejection, thereby allowing the development and survival of transplanted tissue. However, OTX still presents important ethical issues, particularly related to animal welfare and rights (Rollin, 2020).

This review discusses OTX as a pivotal tool for studying and optimizing OTCT methods, serving as a promising model to predict and advance fertility restoration. We describe and analyze the significant milestones and breakthroughs achieved in this domain. Additionally, we examine the potential applications and

future directions of OTX, providing insights into its evolving role in the realm of reproductive medicine.

Mouse models for OTX

In practice, human OTX is usually undertaken in mouse strain models. The primary challenge in OTX is overcoming graft rejection by the recipient animal's immune system. To address this, immunodeficient mouse models have become the pillar of OTX research: namely, the immunodeficient mouse model (nude); severe combined immunodeficient (SCID) mice; non-obese diabetic (NOD) mice; and the crossbred NOD-SCID mice (see Table 1). The NOD-SCID mouse model has been considered to have a more severe degree of immune deficiency than previously mentioned mouse models (Shultz et al., 1995) (Fig. 1).

While nude mice are commonly preferred for short-term xenografting experiments (Dath et al., 2010; Amorim et al., 2012), SCID mice are typically favored for long-term xenografting studies (Amorim et al., 2011; David et al., 2012). Nevertheless, research on OTX has shown that NOD and SCID mice yield superior outcomes in terms of antral follicle formation and maintenance of ovarian tissue morphology (Terada et al., 2008).

Studies on OTX and murine models have a focus on the efficacy of different mouse strains in enhancing follicular viability and detecting malignant cells or assessing pathological conditions within transplanted ovarian tissue. Recently, γ-irradiated NMRI mice have been used for OXT, yielding significant findings related to follicular apoptosis after vitrification, effects of ischemia during grafting procedures, and alterations in the expression of genes associated with follicular activity (Jafarabadi et al., 2015; Mofarahe et al., 2020). Furthermore, other mouse strains, such as NOD SCID gamma mice (Sanchez et al., 2009), hold potential for further research, particularly in studying neoplastic cell dissemination and evaluating residual disease within ovarian tissue.

Although immunodeficient mice offer a valuable model for OTX, they are not a perfect human replica. Comparing follicle populations before and after OXT, significant changes in gene expression and epigenetic patterns were described, likely due to xenograft microenvironment differences, with potential implications for medical research (Man et al., 2020). Several key differences can influence human follicle growth in mice, including the hormonal milieu of the recipient, the transplantation site within the recipient, and even species-specific differences in growth factors and signaling pathways crucial for healthy folliculogenesis. According to Man et al. (2023), these xenograft-related alterations impact directly on follicular parameters, such as growth, mobilization, and volume, and even influence the development of neighborhood and in growing follicles.

Researchers are actively investigating the ideal grafting site. Numerous transplantation sites have been explored, including intraperitoneal, inside the ovarian bursa, under the kidney capsule, intramuscular, and subcutaneous (Van Eyck et al., 2009; Cacciottola et al., 2018; Manavella et al., 2018a,b; Shapira et al., 2018; Lunding et al., 2020; Lee et al., 2021) (Fig. 2). While the studies have considered several key elements, including the exponential growth of human follicles, oxygen tension, and vascularization (Dath et al., 2010), the optimal site remains under debate (Dath et al., 2010; Ruan et al., 2019) (Table 1).

Preclinical studies and experimental findings

Research on OTX has produced over 40 original studies in the last decade. These studies focus on six main areas: (i) ovarian function and physiology, (ii) effects of exogenous factors on follicle growth, (iii) revascularization of cryopreserved and transplanted tissues, (iv) risk of malignant cell reintroduction, (v) development of improved cryopreservation and transplantation protocols, and (vi) the impact of chemotherapeutic agents on ovarian follicles.

Ovarian function and physiology

A significant challenge in OTCT is the considerable loss of follicles during cryopreservation and subsequent transplantation. This loss is primarily attributed to damage from freezing and ischemia/reperfusion during the initial stages after transplantation (Baird et al., 1999; Roness and Meirow, 2019). Researchers are working to understand the molecular pathways governing ovarian function and follicle development to develop strategies that improve blood flow and reduce tissue damage. Studies have highlighted the importance of the oxygen environment in OTX, as hypoxia can negatively impact follicular development and vascularization (Van Eyck et al., 2009).

Effects of exogenous factors on follicle growth

Exploring signaling pathways that influence follicle activation and growth is an emerging area of research. Pathways such as the Hippo and PTEN/PI3K/Akt cascades play a role in regulating early follicle activation within the ovary (Lunding et al., 2020). Fragmentation of ovarian tissues has been shown to disrupt the Hippo pathway via yes-associated protein, which prompts early follicle activation and stimulates the PTEN/PI3K/Akt signaling pathway (Grosbois et al., 2020). However, studies indicate that xenotransplantation of fragmented human ovarian tissue into mice does not improve follicle survival, suggesting that fragmentation may not be an effective approach for preserving ovarian function or enhancing follicle viability in transplantation settings (Lunding et al., 2020) (Table 1).

Revascularization of cryopreserved and transplanted tissues

Revascularization is critical for the survival and function of grafted ovarian tissue. Mechanical methods, such as puncturing samples or using an yttrium aluminum garnet laser to remove the ovarian surface epithelium, have not effectively improved revascularization or follicle survival (Mamsen et al., 2021; Olesen et al., 2023). In contrast, chemical approaches have shown more promise. Studies indicate that treatments with antioxidants, glutathione, ulinastatin, and vitamin E can reduce follicle loss and mitigate ischemia, oxidative stress, and inflammation in post-human OTX mice (Li et al., 2021). Additional investigations have demonstrated the benefits of anti-apoptotic agents like Z-Val-Ala-Asp-fluoromethylketone and sphingosine-1-phosphate (S1P), which improve follicle survival and vascularization in OTX models (Henry et al., 2016; Lee et al., 2021). Co-transplantation of mesenchymal stem cells with ovarian tissue has also yielded promising results, enhancing angiogenesis, follicle viability, and potentially prolonging the longevity of transplanted tissue (Manavella et al., 2018a,b; Cacciottola et al., 2020) (Table 1).

Risk of malignant cell reintroduction

The potential reintroduction of malignant cells is a critical concern in OTCT, particularly for patients with a history of cancer. OTX has emerged as a valuable model to investigate the behavior of potentially cancerous cells and to detect malignancy within the tissue (Table 1). For example, a study by Bastings et al. (2013) analyzed the risks of cancer recurrence in ovarian tissue

Table 1. Original articles on ovarian tissue xenotransplantation (OTX) in mice published in the last 10 years.

Preclinical study approach	Major outcome	Animal model	Grafting site	Xenografting duration	Reference
Strategies to improve ovarian tissue transplantation outcomes	Mechanical-induced angiogenesis using needle punctures prior to transplantation did not enhance ovarian graft revascularization	Nude mice	Subcutaneous	3, 6, and 10 days	Olesen et al. (2023)
	Co-transplantation of hypoxia-preconditioned human umbilical cord mesenchymal stem cells with ovarian tissue enhanced vascularization of ovarian grafts yielding a higher follicle survival rate and decreased apoptosis	Nude mice	Under the kidney capsule	3 and 7 days	Cheng et al. (2022)
	Both sides of ovarian cortical strips could be transplanted with no significantly different data on angiogenesis biomarkers	Nude mice	Subcutaneous	8 days and 8 weeks	Kristensen et al. (2022)
	ASCs positively affect ovarian reserve by safeguarding primordial follicles and maintaining their quiescence via PI3K/Akt pathway modulation	Nude mice	Intraperitoneal	3 and 10 days	Cacciottola et al. (2021)
	Intraperitoneal administration of Z-VAD-FMK preserved primordial follicles and the angiogenesis process	SCID mice	Intramuscular	4 weeks	Lee et al. (2021)
	Intravenous administration of glutathione and ulinastatin reduces follicle loss	SCID mice	Subcutaneous	1,3,7,14,28,56 and 85 days	Li et al. (2021)
	Mechanical-induced angiogenesis using YAG laser treatment did not improve ovarian graft revascularization	Nude mice	Subcutaneous	8 days and 8 weeks	Mamsen et al. (2021)
	NAC administration reduced ischemia-reperfusion injury and increased follicle survival	Nude mice	Subcutaneous	8 and 29 days	Olesen et al. (2021)
	ASCs before OTX increased primordial follicles and improved follicle distribution in grafts, potentially prolonging ovarian tissue lifespan	SCID mice	Intraperitoneal	6 months	Cacciottola et al. (2020)
	Administration of Z-VAD-FMK improved the preservation of primary follicles and reduced global apoptosis	SCID mice	Intramuscular	3 days or 3 weeks	Fransolet et al. (2019)
	ASCs differentiate into human vessels and stimulate VEGF secretion when co-transplanted with human ovarian tissue	SCID mice	Intraperitoneal	7 days	Manavella et al. (2019)
	Recombinant AMH administration reduced apoptosis and cellular activation	Nude mice	Intramuscular	7 days	Detti et al. (2018)
	When transplanted into mouse peritoneum within a fibrin matrix, human ASCs at high concentrations led to significant increases in human vessel area	SCID mice	Intraperitoneal	14 days	Manavella et al. (2018a)
	Increased rates of oxygenation and vascularization of ovarian tissue, along with increased follicle survival rates, were observed in the early post-grafting period	SCID mice	Intraperitoneal	7 days	Manavella et al. (2018b)
	Local administration of sustained-release bFGF induced neovascularization in ovarian tissue	SCID mice	Subcutaneous	6 weeks	Tanaka et al. (2018)
	New and known genes were found to be key players in tissue restructuring and angiogenesis post-human ovarian tissue transplantation	Nude mice	2 and 7 days	Intraperitoneal	Van Langendonckt et al. (2014)
Assessment of cryopreservation protocols	Passive slow freezing commercial container could maintain follicle growth in grafted tissues comparable to controlled slow rate freezing	Nude mice	Intraperitoneal	4 and 6 weeks	Lierman et al. (2021)
	Human ovarian cortical tissue could be re-frozen and re-thawed without deleterious effects on follicle ultrastructure	SCID mice	Intraperitoneal	21 days	Hossay et al. (2020)
	Vitrification method showed no harmful effect on follicle growth and expression of genes related to folliculogenesis	γ-irradiated NMRI mice	Subcutaneous	2 weeks	Mofarahe et al. (2020)
	LPA supplementation to human ovarian tissue culture medium enhanced follicular survival and development by modulating the transcription of *BCL2* and *BAX* genes towards an anti-apoptotic balance	γ-irradiated NMRI mice	Intramuscular	2 weeks	Mohammadi et al. (2020)
	Slow freezing yielded better results in terms of follicle survival and growth compared to vitrification	SCID mice	Intramuscular	4 weeks	Lee et al. (2019)
	Higher cryoprotectant concentration and lower seeding temperature did not improve follicle survival	SCID mice	Intraperitoneal	3 weeks	Gallardo et al. (2018)

(continued)

Table 1. Continued

Preclinical study approach	Major outcome	Animal model	Grafting site	Xenografting duration	Reference
	A silver-based closed vitrification system showed promise as a carrier for cryopreserving human ovarian tissue	SCID mice	Subcutaneous	3, 7, 14, and 21 days	Xiao et al. (2017b)
	The presence of medulla in ovarian pieces enhances the post-thaw development of cryopreserved human ovarian tissue	SCID mice	Subcutaneous	45 days	Isachenko et al. (2016)
	Vitrification has an impact on follicle apoptosis at the mRNA level	γ-irradiated NMRI mice	Intramuscular	30 days	Jafarabadi et al. (2015)
	bFGF improved the quality of human ovarian tissue	SCID mice	Subcutaneous	7 days	Kang et al. (2016)
	Short-term xenografts (1-4 weeks) support better initial viability and potential for follicle development compared to long-term (14-22 weeks)	NSG mice	Intramuscular	1-52 weeks	Man et al., 2023
Risk of malignant cell reintroduction	Central nervous system tumors (astrocytoma, ependymoma, glioblastoma, or medulloblastoma) were negatively detected either from non-grafted or grafted ovarian cortical strips	SCID mice	Intraperitoneal	22 weeks	Nguyen et al. (2021)
	Newer histological and molecular techniques hold promise as potential alternatives to xenotransplantation to evaluate the viability and safety of cryopreserved human ovarian tissue	NOD-SCID mice	Subcutaneous	2 and 5 weeks	Gudlevičienė et al. (2020)
	Glandular lesions were observed in xenografted ovarian tissue from 9% of borderline ovarian tumor patients	SCID mice	Intraperitoneal	5 months	Masciangelo et al. (2018)
	No leukemia cell presence was observed post-xenotransplantation of ovarian tissues from acute myeloid leukemia patients	SCID mice	Intraperitoneal	6 months	Shapira et al. (2018)
Activation of follicle growth	YAP-induced by ovarian fragmentation did not activate *in vitro* growth	Nude mice	Subcutaneous	6 weeks	Lunding et al. (2020)
	S1P did not succeed in promoting follicle activation or growth *in vivo*	Nude mice	Subcutaneous	6 weeks	Pors et al. (2020)
	No positive effects of ovarian tissue cutting and *in vitro* activation approach	SCID mice	Intraperitoneal	4 weeks	Dolmans et al. (2019)
Effect of cancer treatment on follicles	Alkylating agents showed harmful effects on ovarian tissue	SCID mice	Subcutaneous	22 weeks	Nurmio et al. (2022)
Protection against chemotherapy drugs	S1P prevented primordial follicle apoptosis after cyclophosphamide application	Nude mice	Subcutaneous	2 weeks	Meng et al. (2014)
Transportation protocol	Cooling at 5°C for 24 h decreased translocation of phosphatidylserine	SCID mice	Subcutaneous	45 days	Isachenko et al. (2015)
Assessment of the follicle basement membrane	Grafting induces maturation of the basement membrane in prepubertal follicles	SCID mice	Intraperitoneal	5 months	Philippart et al. (2021)
Best xenografting site in mouse	Inside the ovarian bursa yielded the best results	Nude mice	Inside ovarian bursa or subcutaneous	1.5 and 2.5 months	Ruan et al. (2019)
Effect of graft host on human ovarian tissue	Significantly higher proportion of growing follicles in the aseptically wounded male mice group	NOD-SCID mice	Subcutaneous	1, 4, 8 and 10 weeks	Luo et al. (2017)
Follicle development after xenografting	Repetitive oocyte retrieval cycles can be achieved without hormone stimulation after OTX	SCID mice	Intramuscular	159 days	Raffel et al. (2017)
	Activation of primordial follicles may impair graft longevity	SCID mice	Subcutaneous	4 and 12 weeks	Ayuandari et al. (2016)
	Oocytes collected from xenografted ovarian tissue can be *in vitro* matured	SCID mice	Intramuscular	122 days	Lotz et al. (2014a)
	Human primordial follicles can be matured to MII oocytes with or without hormone stimulation	SCID mice	Intramuscular	4 or 24 weeks	Lotz et al. (2014b)

AMH, anti-Müllerian hormone; ASCs, adipose tissue-derived stem cells; bFGF: basic fibroblast growth factor; LPA, lysophosphatidic acid; NAC: N-acetylcysteine; NMRI, Naval Medical Research Institute; SCID, severe combined immunodeficient; S1P, sphingosine-1-phosphate; VEGF, vascular endothelial growth factor; YAP, yes-associated protein; Z-VAD-FMK, benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone.

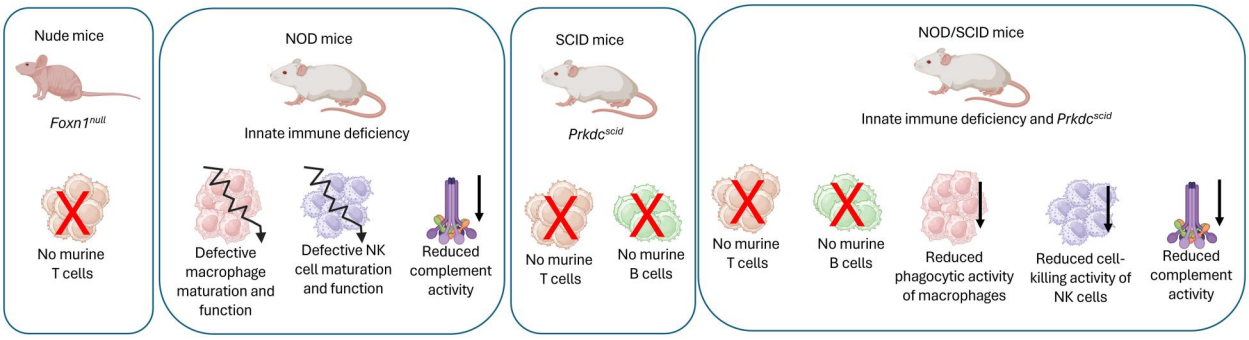


Figure 1. Characteristics of different immunodeficient mice. Nude mice lack T cells due to Foxn1 mutation. SCID mice lack T and B cells due to Prkdc mutation. NOD mice combined with SCID mice produce NOD/SCID mice, which lack T and B cells and have reduced phagocytic activity of macrophages, reduced cell-killing activity of NK cells, and reduced complement activity. Reprinted and adapted from Cheng et al. (2022) with permission from the Creative Commons (www.creativecommons.org). Created by BioRender.com. SCID, severe combined immunodeficient; NOD, non-obese diabetic; NK, natural killer.

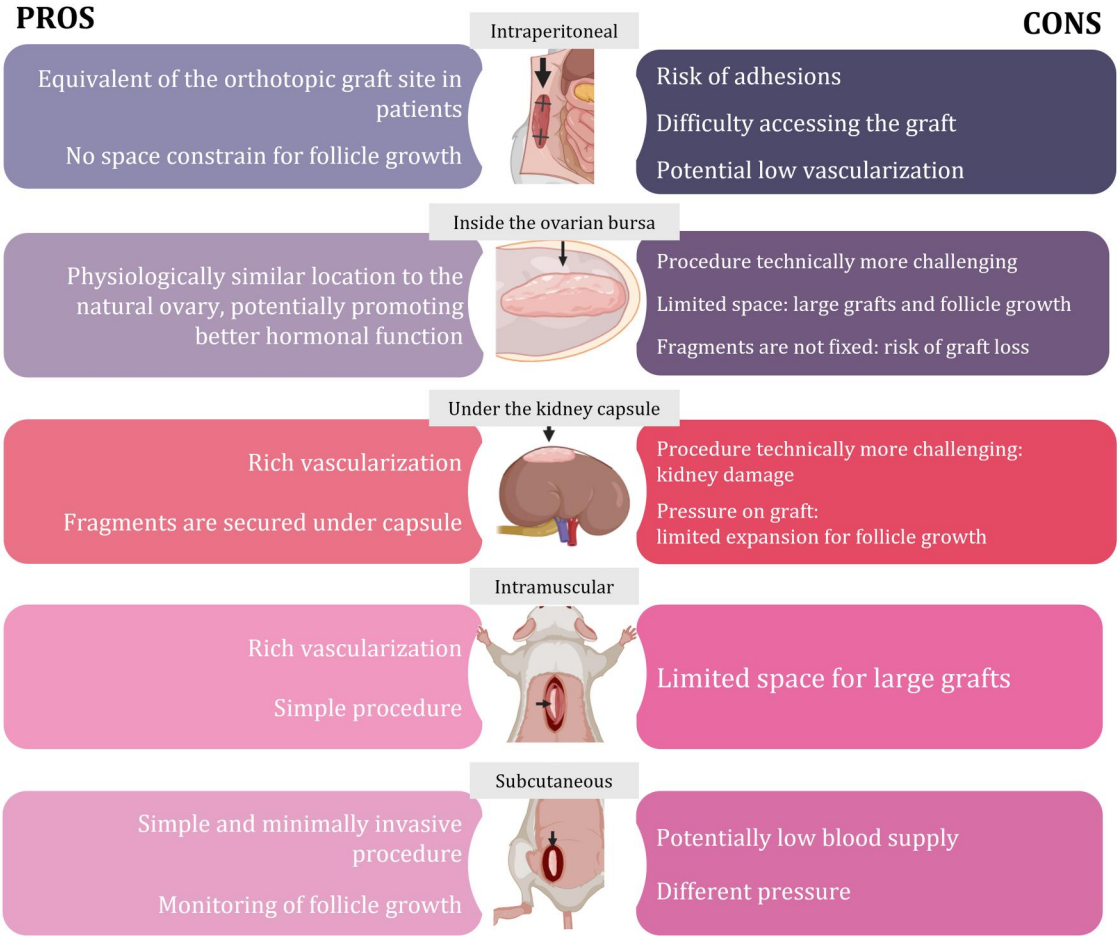


Figure 2. Advantages and disadvantages of the different sites for human OTX. From top to bottom: intraperitoneal, inside the ovarian bursa, under the kidney capsule, intramuscular, and subcutaneous. Created by premast.com and BioRender.com. OTX, ovarian tissue xenotransplantation.

transplanted in cancer survivors, identifying leukemia as a major concern. OTX from acute myeloid leukemia patients into SCID mice for 6 months did not show malignant cells in histological analysis, though longer follow-up may be required to assess full recurrence risk (Shapira et al., 2018). Additional studies using OTX have explored risks related to other cancers, such as borderline ovarian tumors, central nervous system tumors, and breast cancer, with some findings confirming the importance of thorough preimplantation screening (Masciangelo et al., 2018; Nguyen et al., 2021) (Table 1).

Development of improved cryopreservation and transplantation protocols

Improving cryopreservation protocols is essential for maintaining high-quality post-thawed and transplanted ovarian tissue. Research has shown that slow freezing can better preserve follicle survival and growth compared to vitrification, although both methods continue to be explored (Ting et al., 2011; Xiao et al., 2017; Lee et al., 2019; Mofarahe et al., 2020). Cost-effective passive slow-freezing techniques have shown effectiveness similar to programmable cooling rate devices in promoting follicle

growth in immunodeficient mice (Lierman et al., 2021). Direct freezing methods are also being considered to minimize mechanical injuries and challenges associated with crystallization and cooling adjustments, with promising results (Maffei et al., 2013; Arav and Patrizio, 2019). Efforts to optimize cryoprotectant protocols have led to testing additives such as lysophosphatidic acid and basic fibroblast growth factor, both of which show promise in enhancing follicle survival by creating an anti-apoptotic environment (Kang et al., 2016; Mohammadi et al., 2020).

Impact of chemotherapeutic agents on ovarian follicles

Protecting ovarian tissue from the harmful effects of chemotherapy, especially from alkylating agents, is crucial for fertility preservation. S1P has shown potential in preventing follicle death induced by chemotherapy, highlighting it as a promising therapeutic molecule (Li et al., 2014; Meng et al., 2014). Additionally, studies on xenografting have demonstrated that chemotherapy drugs can have significant adverse effects on ovarian tissue. For instance, Nurmio et al. (2022) confirmed the negative impact of chemotherapy agents on ovarian tissue after xenografting, emphasizing the need for protective strategies like S1P to support follicle survival and function under chemotherapy exposure (Table 1).

Challenges and future perspectives

OTX for research purposes

While OTX has emerged as a valuable tool in refining OTCT techniques, it faces several hurdles that need to be addressed for its continued development and potential future application in human fertility restoration.

Regarding immune rejection, OTX relies heavily on immunodeficient mice, particularly SCID strains (Table 1), to overcome graft rejection by the recipient's immune system. These mice lack functional T and B lymphocytes, allowing for human tissue survival and follicle development. However, even immunodeficient mice retain some residual immune activity. This can negatively impact long-term graft survival and potentially bias the results in terms of follicle growth and function. The immune microenvironment influences folliculogenesis by crosstalk between immune cells, cytokines, growth factors, and follicle formation (reviewed by Dai et al., 2023). Ideally, a perfect model would closely mimic the human immune system to provide the most accurate assessment of OTCT outcomes. However, achieving this balance remains a challenge.

We must also consider the species-specific differences between humans and mice. The hormonal environment within the recipient mouse can influence human follicle development differently compared to the human body. Additionally, species-specific factors, such as growth factors and signaling pathways, can play a role in follicle health. Research should address this challenge by carefully considering the limitations of the model system and designing experiments that account for these biological discrepancies. Additionally, focusing on fundamental processes related to follicle growth and survival may offer more broadly applicable knowledge (Man et al., 2020).

OTX offers a valuable platform to assess the risk of reintroducing malignant cells with OTCT, particularly for patients with a history of cancer. By transplanting ovarian tissue from these patients into mice, researchers can evaluate the presence of microscopic tumors or pre-cancerous lesions that might not be detectable through standard histological examination. This helps determine if OTCT is a safe option for these patients without risking their health by reintroducing cancer cells. However, newer techniques, such as advanced histological, molecular, and omics approaches (e.g., transcriptomics, proteomics, and metabolomics), are emerging that may offer a more ethical and potentially more efficient approach to patient safety evaluation.

OTX for clinical application

Researchers have successfully retrieved human ovarian follicles at various developmental stages (antral, germinal vesicle, and metaphase II (MII) oocytes) following OTX (Amorim et al., 2011; Lotz et al., 2014a; Raffel et al., 2017). Notably, these MII oocytes displayed normal size and morphology, suggesting their potential for maturation (Dittrich et al., 2015). However, human embryos from MII oocytes inseminated by IVF cannot be established due to safety and ethical concerns (Dittrich et al., 2015). The primary concern lies in the potential transmission of zoonotic pathogens, particularly prions and endogenous retroviruses, from the animal host to humans (Dittrich et al., 2015). Furthermore, ethical frameworks and legal regulations of using oocytes derived from xenotransplanted tissue for ART remain largely unexplored. However, the specific ethical considerations surrounding *in vitro* maturation and IVF using oocytes retrieved from xenotransplanted ovarian tissue have not been comprehensively addressed.

Ethical concerns regarding the use of animals

It is important to consider ethical concerns regarding animal welfare and rights that arise by the use of animals in OTX research. The public closely monitors the development of novel therapeutic treatments and medical products, and the use of animals in the testing process can raise questions about the ethics and necessity of such research. Therefore, it is crucial to actively explore alternative models, such as using microfluidic chip platforms and 3D culture systems (Xiao et al., 2017; Thuwanut et al., 2021, 2022; Dadashzadeh et al., 2023).

Conclusion

OTCT has witnessed significant advancements in recent years, offering hope for individuals facing infertility due to various medical conditions or treatments. Despite the remarkable progress achieved in restoring reproductive function and hormonal balance through ovarian tissue transplantation, several challenges persist, necessitating further research and innovation.

The utilization of xenotransplantation as a tool for investigating ovarian tissue transplantation techniques and optimizing protocols has been instrumental in overcoming obstacles such as follicle loss and the potential reintroduction of malignant cells. Through experimentation in immunodeficient mouse models, researchers have gained valuable insights into follicle development, tissue revascularization, and the effects of chemotherapy on ovarian function. These findings have not only enhanced our understanding of ovarian biology but also paved the way for the development of novel preservation and treatment strategies. However, translating research findings from animal models to clinical applications presents its own set of challenges, including species-specific differences and ethical considerations. While xenotransplantation offers a valuable platform for assessing the safety and efficacy of ovarian tissue transplantation, alternative methods that minimize animal use while maximizing clinical relevance are actively being explored.

As we continue to unravel the complexities of ovarian tissue transplantation and strive to address ethical concerns surrounding animal welfare, collaboration between researchers, clinicians, ethicists, and policymakers becomes increasingly vital. By

leveraging innovative technologies and ethical frameworks, we can advance the field of reproductive medicine and offer hope to individuals seeking to preserve their fertility and overcome infertility challenges.

Data availability

No new data were generated or analyzed in support of this research.

Authors' roles

All authors participated in writing and reviewing the article and contributed to the final draft.

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Conflict of interest

The authors declare no competing interests.

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