Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in
Endocrine and Metabolic Research

Review

From isolated follicles to the artificial ovary: Why and how?

Marie-Madeleine Dolmans[1,2]

Abstract

Ovarian tissue cryopreservation is a method of fertility preservation implemented before gonadotoxic treatment. This article will focus on the specific technique of follicle isolation with the goal of creating an artificial ovary, the why and how of which will be discussed. The purpose of the artificial ovary is to avoid reintroduction of malignant cells. The first part of the article will review pathologies at risk, whereas the second part will elaborate on practical elements of the technique, with safe human follicle isolation crucial as a first step before embedding follicles inside a scaffold.

Addresses

[1] Pôle de Gynécologie, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, 1200 Brussels, Belgium
[2] Gynecology Department, Cliniques Universitaires Saint Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium

Corresponding author: Dolmans, Marie-Madeleine (marie-madeleine.dolmans@uclouvain.be)

Current Opinion in Endocrine and Metabolic Research 2021, **18**:62–68

This review comes from a themed issue on **Regulation of Ovarian Function**

Edited by **Norah Spears** and **Kate Hardy**

For complete overview of the section, please refer the article collection - Regulation of Ovarian Function

Available online 9 February 2021

https://doi.org/10.1016/j.coemr.2021.01.003

2451-9650/© 2021 Elsevier Ltd. All rights reserved.

Keywords

Chemotherapy, Fertility preservation, Ovarian tissue cryopreservation, Minimal disseminated disease, Malignant cell, Isolated follicles, Scaffold, Matrix.

Introduction

Demand for fertility preservation for oncological, nononcological, and social reasons has seen a significant increase in recent years, with embryo cryopreservation, mature oocyte cryopreservation, and ovarian tissue cryopreservation now all endorsed by the American Society for Reproductive Medicine as appropriate means [1]. Among these methods, oocyte vitrification provides the highest yield for women with benign diseases, those seeking fertility preservation for personal reasons, and also cancer patients in a position to postpone their treatment. In prepubertal girls and women whose cancer treatment cannot be delayed, however, ovarian tissue cryopreservation is expressly indicated [2]. This technique of ovarian tissue freezing followed by autotransplantation has already yielded well over 200 healthy live births [3]. The aim of this overview is to discuss indications for new techniques in the field of fertility preservation. We will focus specifically on the technique of follicle isolation with the goal of creating an artificial ovary. The why and how of the artificial ovary will also be discussed.

Why

There is great concern about the possible presence of malignant cells in frozen ovarian tissue, but not all cancers have the same risk (See Table 1). It is absolutely vital that we rule out any potential contamination to avoid reimplantation of malignant cells, and hence recurrence of the primary disease. In our reviews, we considered three pathologies at particularly high risk of the presence of malignant cells in the ovary: leukemia, Burkitt lymphoma, and neuroblastoma [4,5]. These pathologies will be reviewed in greater detail.

Leukemia

Leukemia is one of the most common cancers affecting children and adolescents [5], and also one of the most curable, with survival rates exceeding 80%. Our team [6] investigated whether leukemic cells were present in ovarian tissue frozen during the active phase of the disease from both chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) patients by polymerase chain reaction (PCR) and xenografting to immunodeficient mice. PCR revealed contaminated ovarian tissue issuing from 70% of ALL patients and 33% of CML patients. After xenotransplantation, none of the CML-grafted mice developed the disease, whereas almost 50% of those grafted with ALL tissue were found to be invaded. On the contrary, Greve analyzed ovarian tissue from leukemia patients in remission and found that ovarian tissue from leukemia patients in complete remission does not contain viable malignant cells capable of transmitting the disease [7].

Lymphomas

a) Non-Hodgkin's lymphoma

No ovarian involvement was found by histology in the few studies conducted on non-Hodgkin's lymphoma

Table 1

Risk of ovarian metastasis according to cancer type, From *Dolmans and Masciangelo, Minerva Gynecologica, 2018.*

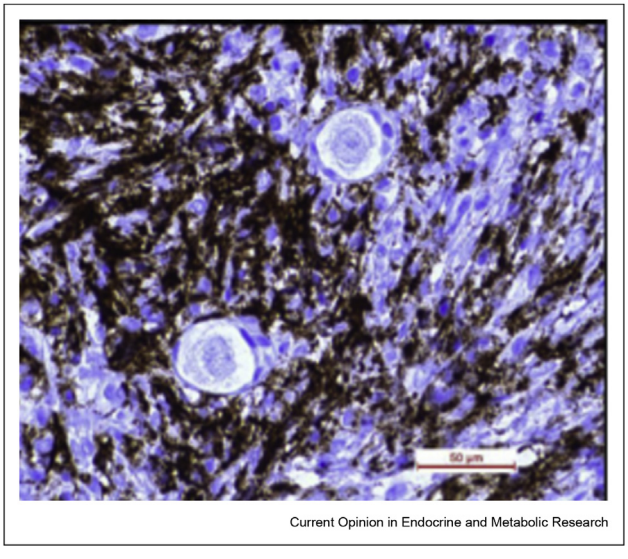
High risk (>10%)	Moderate risk (2–10%)	Low risk (0–2%)
Leukemia	Breast cancer, advanced stage	Breast cancer, early stage
Neuroblastoma	Colorectal cancer	Squamous cell carcinoma of the cervix
Burkitt lymphoma	Adenocarcinoma of the cervix	Hodgkin lymphoma
	Non-Hodgkin lymphoma	Rhabdomyosarcoma
	Ewing sarcoma	Soft tissue sarcoma
	Ovarian cancer	
	Borderline ovarian tumor	

(NHL) [8,9]. By performing xenografting experiments with ovarian tissue from five NHL patients, Kim et al. were unable to prove disease recurrence [8], but Meirow did report one case of a solid pelvic mass in a 33-year-old patient with high-grade B-cell NHL [9]. Dolmans et al. also detected malignant cells in the cryopreserved ovarian tissue of two NHL patients by histology and anti-CD20 immunohistochemistry [4] (Figure 1). Hence, caution is required in case of NHL, especially when patients present in the leukemic phase of the disease commonly associated with cystic ovarian masses [9].

b) Burkitt lymphoma

Burkitt lymphoma is an infrequent but very aggressive B-cell form of NHL. It often involves the lymph nodes, but the central nervous system, bowel, jaw, ovaries, and kidneys may also be affected. Burkitt lymphoma typically responds well to high-dose chemotherapy, with excellent recovery rates [10]. The ovary is the most common site of NHL in the female genital tract and Burkitt lymphoma appears to account for approximately 19% of adnexal lymphomas [11,12]. Grafting of ovarian tissue is not deemed safe in patients with Burkitt lymphoma because of its aggressiveness and likely ovarian involvement [4,13].

Figure 1



Ovarian fragment from an NHL patient. Massive invasion of the cortex by NHL cells is evidenced by anti-CD20 immunohistochemistry. Adapted from Dolmans et al., F&S, 2013.

Neuroblastoma

Neuroblastoma is the most frequent extracranial solid tumor in young children, and often metastasizes. Indeed, over 60% of metastatic neuroblastomas show circulating malignant cells on diagnosis [14–16]. Treatment includes high-dose chemotherapy and hematopoietic stem cell transplantation, leading to a significant risk of infertility by premature menopause occurring in more than 80% of subjects. As these patients are usually prepubertal, ovarian tissue cryopreservation is the only available option to preserve their fertility [2,17]. Neuroblastoma very frequently spreads to the ovaries, exhibiting high rates of circulating malignant cells on diagnosis, as stated earlier. This clearly presents a threat of disease recurrence after ovarian tissue reimplantation. However, no systematic analyses have yet been published on minimal disseminated disease in ovarian tissue from patients diagnosed with neuroblastoma, although preliminary results in our department show the risk is minimal [18].

In conclusion, the pathology that carries the highest risk of reimplanting malignant cells when transplanting ovarian tissue is acute leukemia, so ovarian tissue transplantation is not recommended in these patients. Nevertheless, cryopreservation of ovarian tissue at the time of disease remission, combined with thorough tissue evaluation using sensitive techniques, can be contemplated in some instances, having enabled safe and successful tissue reimplantation in a patient with acute myeloid leukemia [19]. This means that those at risk of ovarian metastasis should not give up hope of ever giving birth. Indeed,

techniques are constantly evolving and there are already two existing options for these leukemia patients. If safety tests reveal a risk, an option to circumvent disease recurrence with frozen tissue, could be in-vitro maturation [20]. The second option is isolating and purging individual follicles that can subsequently be grafted back to patients inside the so-called artificial ovary [21].

How

The main question that needs to be addressed is how to assemble the artificial ovary.

Isolation

The first step in creating an artificial ovary requires isolation of a large number of good-quality primordial follicles. Because human ovarian cortex is fibrous, the most effective way to obtain a high follicle yield and sufficient follicle quality involves a combination of mechanical and enzymatic digestion of the tissue [22]. We optimized our isolation protocol [23] in order not to expose fully isolated follicles for too long, as protracted exposure to enzymes could damage their basement membrane and result in their death. Moreover, to increase the number of isolated follicles, the remaining incompletely digested fragments of ovarian tissue would then be digested in fresh enzymatic solution [24]. This filtration-digestion step was in fact repeatedly carried out until complete digestion of all the ovarian fragments. By immersing the remaining fragments in fresh enzymatic solution every 30 min, we successfully digested all types of ovarian tissue, from the softest to the hardest. After encapsulation in fibrin and xenotransplantation, this technique allowed us to retrieve hundreds of follicles (ranging from 100 to 1000, depending on the size of the biopsy and original follicle density) to embed inside the artificial ovary without affecting their quality [24].

Safe isolation

After isolation, however, follicles and cells were both found in the suspension, so during pick-up of isolated follicles destined for the artificial ovary, malignant cells could also have been inadvertently retrieved (Figure 2). As the purpose of the artificial ovary is to avoid reintroduction of malignant cells, it is vital that we prevent any contamination of the isolated follicle suspension. To this end, Soares et al [25] developed a washing step, whereby isolated follicles were transferred three times from one medium droplet to another to separate them from surrounding isolated cells. After washing the suspensions three times following this protocol [25], none of the samples (>2300 follicles) exhibited any malignant cells whatsoever and all tested negative for leukemia. This offers leukemia patients genuine hope of having their fertility restored, as well as reassuring them about the safety of the artificial ovary.

Scaffold

In the same way as ovarian tissue, a functioning artificial ovary is intended to restore both endocrine and reproductive functions after transplantation. A number of prototypes have been designed to support follicle development and ovulation, promote female hormone production, and yield embryos and healthy offspring in murine models [26–34]. We could speculate that these results may be extrapolated to human follicles inside similar scaffolds, but we must remember that murine and human ovary microarchitecture and structure differ substantially. Requirements for human follicles can indeed be distinct from those for murine follicles [35,36]. Although studies in humans still lag far behind those in mice, and despite the ethical challenges involved in human reproductive function assessment, studies have proved the feasibility of this approach.

To promote follicle survival and growth after grafting inside the artificial ovary, various conditions must be met to ensure female hormone production and fertilizable mature oocyte development. Although complete follicle isolation is needed, follicle survival and growth depend on cross-talk between follicles and surrounding ovarian cells. For example, ovarian stromal cells are implicated in activating primordial follicles and some stromal cells are recruited to differentiate into theca cells [37,38]. Conversely, endothelial cells govern vascularization in ovarian tissue, vital to transporting paracrine factors, oxygen and nutrients, and removing metabolic waste [39]. In other words, we must recreate an environment that resembles the human ovary as closely as possible.

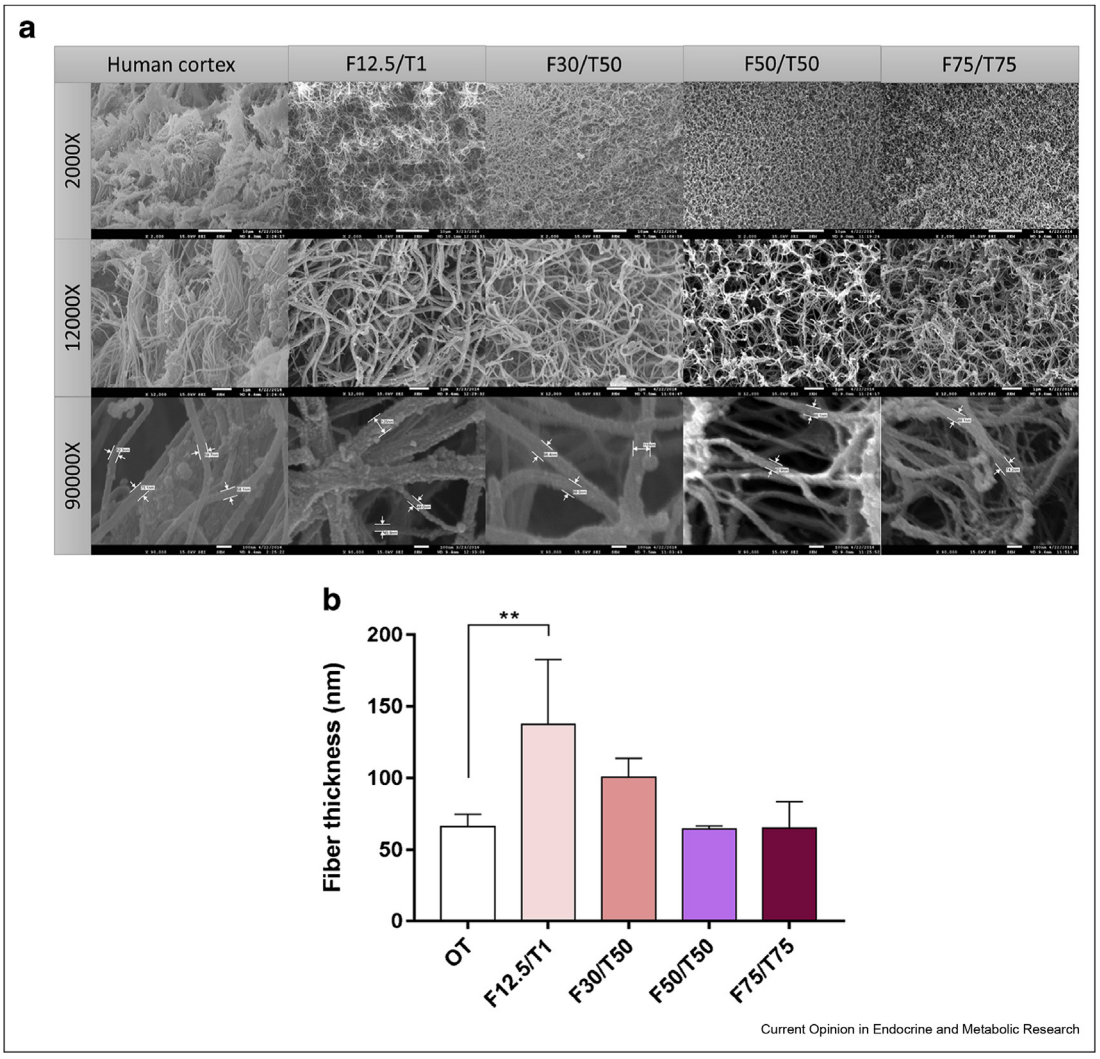
a) Alginate

Alginate, a natural polymer used extensively in tissue engineering, was tested for grafting of isolated preantral follicles, because very few studies have investigated alginate in this context [30,34,40]. After isolation and encapsulation in a 1% alginate scaffold, isolated murine preantral follicles were found to have survived and developed after 7 days of autotransplantation [30]. Interestingly, use of a lower alginate concentration (0.5%) allowed Rios et al [34] to obtain metaphase II oocytes after grafting.

b) Natural matrices and fibrin

The first natural material ever used to graft isolated preantral follicles was collagen [27]. That same year, Gosden [26] reported the birth of mouse pups after grafting of isolated follicles in plasma clots. Based on these successful results with mouse follicles, Dolmans et al. [41,42] encapsulated isolated human preantral follicles in autologous plasma clots and xenotransplanted

Figure 2



Ultrastructure of human ovarian cortex and fibrin formulations. (a) SEM image of human ovarian cortex and fibrin matrix formulations at different magnifications (× 2000; ×12 000; ×90 000). (b) Graphic representation of fiber thickness in human ovarian tissue (OT) and four fibrin formulations. Adapted from Chiti MC et al., JARG, 2018; 35:41–48.

them to immunosuppressed mice. Follicles developed to the antral stage after long-term grafting [41,42].

Successful outcomes of isolated mouse follicle transplantation were also obtained with gelatin and decellularized ovarian tissue [33,43]. Pors et al [44] recently reported that human isolated follicles were able to survive for 3 weeks after seeding inside decellularized ovarian tissue and xenografting.

Our team also tested fibrin, another natural polymer. Short-term (7 days) transplantation of isolated mouse preantral follicles inside fibrin matrices with very low levels of fibrinogen and thrombin led to survival and growth of follicles and yielded recovery rates of 28–35% [45–47]. After a longer (3 weeks) period and inside a stiffer fibrin matrix, isolated mouse preantral follicles developed to the antral stage [48]. The presence of corpora lutea and the decline in follicle-stimulating hormone levels proved restoration of hormone cyclicity.

Fibrin has been combined with other natural polymers (fibrin-collagen and fibrin-alginate) and supplemented with growth factors (vascular endothelial growth factor (VEGF)) an attempt to enhance follicle survival [31]. Surprisingly, after grafting isolated murine preantral follicles inside these different scaffolds, pups were only obtained from fibrin-VEGF clots [31]. Fibrin has also been used along with platelet lysate, a great source of growth factors [49], indicating that such supplementation can

enhance recovery rates after 14 days of isolated mouse follicle transplantation [50].

Different fibrin formulations have also been used to investigate survival and growth of human follicles [24,35]. Fibrin matrices containing 50 mg/ml fibrinogen and thrombin were found to most closely resemble human ovarian cortex in terms of ultrastructure (Figure 2) and rigidity [36]. With this type of matrix, follicle recovery rates of *22%* were achieved after 1 week of xenografting to immunodeficient mice [35].

Conclusion

Ongoing research into a transplantable artificial ovary prototype for human isolated follicles should now be directed at investigating follicle morphometry, growth rates, hormone secretion, molecular markers, and the epigenetic status of oocytes. Knowledge of these aspects is crucial to help us understand how a given matrix can impact follicle survival and development.

Funding

This study was supported by grants from the Fonds National de la Recherche Scientifique de Belgique: Excellence of Science (FNRS–EOS) grant number 5/4/150/5 awarded to Marie-Madeleine Dolmans, FNRS-PDR Convention T.0077.14 to Marie-Madeleine Dolmans.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors thank Mira Hryniuk, BA, for reviewing the English language of the manuscript.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. **Practice Committee of the American Society for Reproductive Medicine Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion**. *Fertil Steril* 2019, **112**:1022–1033.

2. * Donnez J, Dolmans MM: **Fertility preservation in women**. *N Engl J Med* 2017, **377**:1657–1665, https://doi.org/10.1056/NEJMra1614676.
Review on clinical options to preserve fertility.

3. Dolmans MM, Falcone T, Patrizio P: **Importance of patient selection to analyze in vitro fertilization outcome with transplanted cryopreserved ovarian tissue**. *Fertil Steril* 2020, **114**:279–280, https://doi.org/10.1016/j.fertnstert.2020.04.050.

4. Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T: **Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue**. *Fertil Steril* 2013, **99**:1514–1522, https://doi.org/10.1016/j.fertnstert.2013.03.027.

5. * Dolmans MM, Masciangelo R: **Risk of transplanting malignant cells in cryopreserved ovarian tissue**. *Minerva Ginecol* 2018:70436–70443, https://doi.org/10.23736/S0026-4784.18.04233-8.
Recent overview on the risk according to pathology.

6. Dolmans MM, Marinescu C, Saussoy P, Van Langendonckt A, Amorim C, Donnez J: **Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe**. *Blood* 2010, **116**:2908–2914, https://doi.org/10.1182/blood-2010-01-265751.

7. Greve T, Clasen-Linde E, Andersen MT, Andersen MK, Sorensen SD, Rosendahl M, Ralfkiaer E, Andersen CY: **Cryopreserved ovarian cortex from patients with leukemia in complete remission contains no apparent viable malignant cells**. *Blood* 2012, **120**:4311–4316, https://doi.org/10.1182/blood-2012-01-403022.

8. Kim SS, Radford J, Harris M, Varley J, Rutherford AJ, Lieberman B, Shalet S, Gosden R: **Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation**. *Hum Reprod* 2001, **16**:2056–2060, https://doi.org/10.1093/humrep/16.10.2056.

9. Meirow D, Hardan I, Dor J, Fridman E, Elizur S, Ra'anani H, Slyusarevsky E, Amariglio N, Schiff E, Rechavi G, *et al.*: **Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients**. *Hum Reprod* 2008, **23**:1007–1013, https://doi.org/10.1093/humrep/den055.

10. Molyneux EM, Rochford R, Griffin B, Newton R, Jackson G, Menon G, Harrison CJ, Israels T, Bailey S: **Burkitt's lymphoma**. *Lancet* 2012, **379**:1234–1244, https://doi.org/10.1016/S0140-6736(11)61177-X.

11. Kosari F, Daneshbod Y, Parwaresch R, Krams M, Wacker HH: **Lymphomas of the female genital tract: a study of 186 cases and review of the literature**. *Am J Surg Pathol* 2005, **29**:1512–1520, https://doi.org/10.1097/01.pas.0000178089.77018.a9.

12. Perlman S, Ben-Arie A, Feldberg E, Hagay Z: **Non-Hodgkin's lymphoma presenting as advanced ovarian cancer–a case report and review of literature**. *Int J Gynecol Canc* 2005, **15**:554–557, https://doi.org/10.1111/j.1525-1438.2005.15324.x.

13. Sonmezer M, Oktay K: **Fertility preservation in female patients**. *Hum Reprod Update* 2004, **10**:251–266, https://doi.org/10.1093/humupd/dmh021.

14. Burchill SA, Lewis IJ, Abrams KR, Riley R, Imeson J, Pearson AD, Pinkerton R, Selby P: **Circulating neuroblastoma cells detected by reverse transcriptase polymerase chain reaction for tyrosine hydroxylase mRNA are an independent poor prognostic indicator in stage 4 neuroblastoma in children over 1 year**. *J Clin Oncol* 2001, **19**:1795–1801, https://doi.org/10.1200/JCO.2001.19.6.1795.

15. Yáñez Y, Hervás D, Grau E, Oltra S, Pérez G, Palanca S, Bermúdez M, Márquez C, Cañete A, Castel V: **TH and DCX mRNAs in peripheral blood and bone marrow predict outcome in metastatic neuroblastoma patients**. *J Canc Res Clin Oncol* 2016, **142**:573–580, https://doi.org/10.1007/s00432-015-2054-7.

16. Greze V, Brugnon F, Chambon F, Halle P, Canis M, Amiot C, Gremeau AS, Pereira B, Yanez Peralta Y, Tchirkov A, *et al.*: **Highly sensitive assessment of neuroblastoma minimal residual disease in ovarian tissue using RT-qPCR-A strategy for improving the safety of fertility restoration**. *Pediatr Blood Canc* 2017, **64**, https://doi.org/10.1002/pbc.26287.

17. Imbert R, Moffa F, Tsepelidis S, Simon P, Delbaere A, Devreker F, Dechene J, Ferster A, Veys I, Fastrez M, *et al.*: **Safety and usefulness of cryopreservation of ovarian tissue to preserve fertility: a 12-year retrospective analysis**. *Hum Reprod* 2014, **29**:1931–1940, https://doi.org/10.1093/humrep/deu158.

18. Nguyen TYT, Camboni A, Masciangelo R, Donnez J, Dolmans MM: **Investigation of malignant cells in ovarian tissue from a patient with central nervous system primitive neuroectodermal tumor relapse after ovarian tissue transplantation**. *Acta Obstet Gynecol Scand* 2020, https://doi.org/10.1111/aogs.14005 [epub ahead of print].

19. Shapira M, Raanani H, Barshack I, Amariglio N, Derech-Haim S, Marciano MN, Schiff E, Orvieto R, Meirow D: **First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination**. *Fertil Steril* 2018, **9**:48–53, https://doi.org/10.1016/j.fertnstert.2017.09.001.
Case report with example of minimal disease testing before transplantation.

20. McLaughlin M, Albertini DF, Wallace WHB, Anderson RA, Telfer EE: **Metaphase II oocytes from human unilaminar follicles grown in a multi-step culture system**. *Mol Hum Reprod* 2018, **24**:135–142, https://doi.org/10.1093/molehr/gay002.
Most recent progress published in ovarian tissue culture.

21. Dolmans MM, Amorim CA: **Fertility preservation: construction and use of artificial ovaries**. *Reproduction* 2019, **158**:F15–F25, https://doi.org/10.1530/REP-18-0536.
Comprehensive article on artificial ovary development.

22. Chiti MC, Donnez J, Andrade Amorim CA, Dolmans MM: **From isolation of human ovarian follicles to the artificial ovary: tips and tricks**. *Minerva Ginecol* 2018, **70**:444–455, https://doi.org/10.23736/S0026-4784.18.04231-4.

23. Vanacker J, Camboni A, Dath C, Van Langendonckt A, Dolmans MM, Donnez J, Amorim CA: **Enzymatic isolation of human primordial and primary ovarian follicles with Liberase DH: protocol for application in a clinical setting**. *Fertil Steril* 2011, **96**:379, https://doi.org/10.1016/j.fertnstert.2011.05.075. e3–383.e3.

24. Chiti MC, Dolmans MM, Hobeika M, Cernogoraz A, Donnez J, Amorim CA: **A modified and tailored human follicle isolation procedure improves follicle recovery and survival**. *J Ovarian Res* 2017, **10**:71, https://doi.org/10.1186/s13048-017-0366-8.

25. Soares M, Sahrari K, Amorim CA, Saussoy P, Donnez J, Dolmans MM: **Evaluation of a human ovarian follicle isolation technique to obtain disease-free follicle suspensions before safely grafting to cancer patients**. *Fertil Steril* 2015, **104**:672, https://doi.org/10.1016/j.fertnstert.2015.05.021. e2–680.e2.

26. Gosden RG: **Restitution of fertility in sterilized mice by transferring primordial ovarian follicles**. *Hum Reprod* 1990, **5**:117–122, https://doi.org/10.1093/oxfordjournals.humrep.a137132.

27. Telfer E, Torrance C, Gosden RG: **Morphological study of cultured preantral ovarian follicles of mice after transplantation under the kidney capsule**. *J Reprod Fertil* 1990, **89**:565–571, https://doi.org/10.1530/jrf.0.0890565.

28. Carroll J, Gosden RG: **Transplantation of frozen-thawed mouse primordial follicles**. *Hum Reprod* 1993, **8**:1163–1167, https://doi.org/10.1093/oxfordjournals.humrep.a138221.

29. Smith RM, Shikanov A, Kniazeva E, Ramadurai D, Woodruff TK, Shea LD: **Fibrin-mediated delivery of an ovarian follicle pool in a mouse model of infertility**. *Tissue Eng* 2014, **20**:3021–3030, https://doi.org/10.1089/ten.TEA.2013.0675.

30. Vanacker J, Dolmans MM, Luyckx V, Donnez J, Amorim CA: **First transplantation of isolated murine follicles in alginate**. *Regen Med* 2014, **9**:609–619, https://doi.org/10.2217/rme.14.33.

31. Kniazeva E, Hardy AN, Boukaidi SA, Woodruff TK, Jeruss JS, Shea LD: **Primordial follicle transplantation within designer biomaterial grafts produce live births in a mouse infertility model**. *Sci Rep* 2015, **5**:17709, https://doi.org/10.1038/srep17709.
First report on mouse pups after grafting of isolated follicles in fibrin.

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

33. Laronda MM, Rutz AL, Xiao S, Whelan KA, Duncan FE, Roth EW, Woodruff TK, Shah RN: **A bioprosthetic ovary created using 3D printed microporous scaffolds restores ovarian function in sterilized mice**. *Nat Commun* 2017, **8**:15261, https://doi.org/10.1038/ncomms15261.
Mouse isolated follicles seeded in a 3D gelatin scaffold yield live births.

34. Rios PD, Kniazeva E, Lee HC, Xiao S, Oakes RS, Saito E, Jeruss JS, Shikanov A, Woodruff TK, Shea LD: **Retrievable hydrogels for ovarian follicle transplantation and oocyte collection**. *Biotechnol Bioeng* 2018, **115**:2075–2086, https://doi.org/10.1002/bit.26721.

35. Paulini F, Vilela JM, Chiti MC, Donnez J, Jadoul P, Dolmans MM, Amorim CA: **Survival and growth of human preantral follicles after cryopreservation of ovarian tissue, follicle isolation and short-term xenografting**. *Reprod Biomed Online* 2016, **33**:425–432, https://doi.org/10.1016/j.rbmo.2016.05.003.
First report on human follicles xenografted in fibrin matrix.

36. Chiti MC, Dolmans MM, Mortiaux L, Zhuge F, Ouni E, Shahri PAK, Van Ruymbeke E, Champagne SD, Donnez J, Amorim CA: **A novel fibrin-based artificial ovary prototype resembling human ovarian tissue in terms of architecture and rigidity**. *J Assist Reprod Genet* 2018, **35**:41–48, https://doi.org/10.1007/s10815-017-1091-3.

37. Parrott JA, Skinner MK: **Kit ligand actions on ovarian stromal cells: effects on theca cell recruitment and steroid production**. *Mol Reprod Dev* 2000, **55**:55–64, https://doi.org/10.1002/(SICI)1098-2795(200001)55:1<55::AID-MRD8>3.0.CO;2-L.

38. Oktem O, Urman B: **Understanding follicle growth in vivo**. *Hum Reprod* 2010, **25**:2944–2954, https://doi.org/10.1093/humrep/deq275.

39. Dath C, Dethy A, Van Langendonckt A, Van Eyck AS, Amorim CA, Luyckx V, Donnez J, Dolmans M: **Endothelial cells are essential for ovarian stromal tissue restructuring after xenotransplantation of isolated ovarian stromal cells**. *Hum Reprod* 2011, **26**:1431–1439, https://doi.org/10.1093/humrep/der073.

40. Vanacker J, Luyckx V, Dolmans MM, Des Rieux A, Jaeger J, Van Langendonckt A, Donnez J, Amorim CA: **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* 2012, **33**:6079–6085, https://doi.org/10.1016/j.biomaterials.2012.05.015.

41. Dolmans MM, Martinez-Madrid B, Gadisseux E, Guiot Y, Yuan WY, Torre A, Camboni A, Van Langendonckt A, Donnez J: **Short-term transplantation of isolated human ovarian follicles and cortical tissue into nude mice**. *Reproduction* 2007, **134**:253–262, https://doi.org/10.1530/REP-07-0131.

42. Dolmans MM, Yuan WY, Camboni A, Torre A, Van Langendonckt A, Martinez-Madrid B, Donnez J: **Development of antral follicles after xenografting of isolated small human preantral follicles**. *Reprod Biomed Online* 2008, **16**:705–711, https://doi.org/10.1016/S1472-6483(10)60485-3.

43. Laronda MM, Jakus AE, Whelan KA, Wertheim JA, Shah RN, Woodruff TK: **Initiation of puberty in mice following decellularized ovary transplant**. *Biomaterials* 2015, **50**:20–29, https://doi.org/10.1016/j.biomaterials.2015.01.051.

44. Pors SE, Ramløse M, Nikiforov D, Lundsgaard K, Cheng J, Andersen CY, Kristensen SG: **Initial steps in reconstruction of the human ovary: survival of pre-antral stage follicles in a decellularized human ovarian scaffold**. *Hum Reprod* 2019, **34**:1523–1535, https://doi.org/10.1093/humrep/dez077.

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

46. Chiti MC, Dolmans MM, Lucci CM, Paulini F, Donnez J, Amorim CA: **Further insights into the impact of mouse follicle stage on graft outcome in an artificial ovary environment**. *MHR: Basic Sci Reprod Med* 2017, **23**:381–392, https://doi.org/10.1093/molehr/gax016.

47. Chiti MC, Dolmans MM, Orellana O, Soares M, Paulini F, Donnez J, Amorim CA: **Influence of follicle stage on artificial ovary outcome using fibrin as a matrix**. *Hum Reprod* 2016, **31**:2898, https://doi.org/10.1093/humrep/dew254.

48. Smith RM, Shikanov A, Kniazeva E, Ramadurai D, Woodruff TK, Shea LD: **Fibrin-mediated delivery of an ovarian follicle pool in a mouse model of infertility**. *Tissue Eng* 2014, **20**: 3021–3030, https://doi.org/10.1089/ten.TEA.2013.0675.

49. Lang S, Loibl M, Herrmann M: **Platelet-rich plasma in tissue engineering: hype and hope. European Surgical Research. Europaische Chirurgische Forschung**. *Rech Chirurgicales Eur* 2018, **59**:265–275, https://doi.org/10.1159/000492415.

50. Rajabzadeh AR, Eimani H, Mohseni Koochesfahani H, Shahvardi AH, Fathi R: **Morphological study of isolated ovarian preantral follicles using fibrin gel plus platelet lysate after subcutaneous transplantation**. *Cell J* 2015, **17**:145–152, https://doi.org/10.22074/cellj.2015.521.