Fertility Preservation in Oncological and Non-Oncological Diseases

A Practical Guide

Michael von Wolff
Frank Nawroth
Editors

Springer

Fertility Preservation in Oncological and Non-Oncological Diseases

Michael von Wolff • Frank Nawroth
Editors

Fertility Preservation in Oncological and Non-Oncological Diseases

A Practical Guide

Springer

Editors
Michael von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine
University Women's Hospital of Bern
Bern
Switzerland

Frank Nawroth
Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology
amedes MVZ Hamburg
Hamburg
Germany

ISBN 978-3-030-47567-3 ISBN 978-3-030-47568-0 (eBook)
https://doi.org/10.1007/978-3-030-47568-0

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

This book is dedicated to all those who commit themselves to preserve fertility and spend time, effort and money to further develop or support this area scientifically and clinically.

Foreword

As a gynecologist dividing my time equally between clinical and research activities, I feel I am in a strong position to see the bigger picture when it comes to the complexities of this new and exciting field. I obtained my PhD on ovarian tissue cryopreservation and transplantation back in 2006 and have continued working in this fascinating area ever since. Curiously, from a very early age, I was drawn to the subject of fertility preservation, possibly sparked by my concern when one of my younger brothers was diagnosed with leukemia at the age of 2.

Since 2012, I have been head of the gynecology research laboratory at the Université Catholique de Louvain, Brussels, a globally renowned center. Prior to that, I had the pleasure and privilege of working with Professor J. Donnez, who achieved the first successful frozen-thawed ovarian tissue transplantation to result in a live birth. I will never forget witnessing the birth of little Tamara in 2004 in our hospital, an experience that served to inspire me further.

Much of my professional life is dedicated to helping both cancer and non-cancer patients, preserve their fertility in the face of gonadotoxic treatments they must endure in order to conquer their disease. Indeed, Michael and I are involved in very similar efforts, but in different European countries, so it was inevitable that our paths would eventually cross. Now we find ourselves assuming the responsibility of leading the International Society for Fertility Preservation (ISFP) as vice-president and president, assisted by colleagues from all over the world, a true honor.

I have always appreciated Michael's clinical work and considerable scientific contribution to the field as author of numerous outstanding articles. Therefore, as current president of the ISFP, I am proud to have been asked to write the foreword to this book on fertility preservation and look forward to working with Michael over the coming years in our new roles.

I am sure you will enjoy reading this book, and dipping into it whenever an unfamiliar clinical situation arises. It is a practical guide based on longstanding experience from clinicians, scientific experts, and pioneers in the field, and anything but a boring succession of theoretical articles. It presents fertility preservation in a very hands-on and realistic way, from its clinical application with appropriate directives and instructive guidance, to the profusion of questions frequently raised by patients.

This book will give physicians, nurses, and biologists with an interest in fertility preservation a highly comprehensive overview.

It is written with "Deutsche Grundlichkeit," a guarantee for success.

In recent years, the demand for fertility preservation for oncological and non-oncological indications, as well as personal reasons, has increased dramatically and we anticipate that this trend will continue. Meeting the demand will prove a major challenge, so professionals in the field need to inform and educate themselves on all aspects. This book is a good place to start.

Marie-Madeleine Dolmans, MD, PhD
Cliniques Universitaires Saint-LucUniversité Catholique de Louvain
Institut de Recherche Expérimentale et Clinique (IREC)
Pôle de Recherche en Gynécologie
Brussels
Belgium

Preface

Four years ago, in early 2016, the first edition of this book was published in German. The book was intended to be a practical guide for the indication and implementation of fertility preservation measures for all those who belonged to the German Austrian Swiss network *Ferti*PROTEKT. Several thousand copies were kindly financed, free of charge, by Ferring Pharmaceuticals, Kiel, Germany.

The book was a great success and was soon considered to be the "Bible" of fertility preservation in the German-speaking world.

The publication of a second free edition was a logical consequence. It should continue to be very practice-oriented, but should also be much more comprehensive, describe further diseases, and also address treatment after fertility preservation. Based on these ideas and considerations, a manual with almost double the scope has emerged.

It was obvious that we should make the book available to an even larger and international readership and Springer-Verlag kindly agreed to publish it in English. The contents were internationalized, the structure was adapted, and the contents were translated.

The result is a practical and helpful manual for accompanying patients from their initial presentation for fertility preservation to later follow-up care. The most common oncological and non-oncological diseases are explained, indications for and against fertility preservation measures are presented, fertility preservation techniques are described in practice-relevant manner, and aftercare after fertility preservation measures is dealt with from the treatment of vaginal bleeding during chemotherapy to the treatment of infertility or premature ovarian failure.

Particular emphasis was placed on:

- The fact that practice-oriented data in tabular form, in the form of graphs and flowcharts that support physicians and embryologists in their treatment.
- Chapters being largely similar in structure and cross-referenced to facilitate orientation.
- Recommendations for or against fertility preservation which are based on age or treatment protocols to avoid both under- and over-therapy.

The result is a manual which enables even oncologists, reproductive physicians and biologists who are less familiar with the subject matter to guide patients through the process of fertility preservation counselling, fertility preservation therapy, and aftercare on an evidence-based, but also individual basis.

We thank all authors for their valuable contributions.

Bern, Switzerland — Michael von Wolff
Hamburg, Germany — Frank Nawroth

Contents

Part IV After Fertility Preservation

Contributors

Magdalena Balcerek Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin, Germany

Berlin Institute of Health (BIH), Berlin, Germany

Department of Paediatric Oncology, Haematology and Stem Cell Transplantation, Augustenburger Platz 1, Berlin, Germany

Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Germany

Anke Barnbrock Clinic for Paediatric and Adolescent Medicine, Department of Oncology, Haematology and Haemostaseology, University Hospital Frankfurt/Main, Goethe-University Frankfurt, Frankfurt, Germany

Karolin Behringer Internal Medicine Department I, Hematology and Oncology, University Hospital of Cologne, Cologne, Germany

Anja Borgmann-Staudt Charité – Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin, Germany

Berlin Institute of Health (BIH), Berlin, Germany

Department of Paediatric Oncology, Haematology and Stem Cell Transplantation, Augustenburger Platz 1, Berlin, Germany

Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Germany

Carolin Bürkle Internal Medicine Department III, Hematology and Oncology, University Hospital of Munich, Großhadern, Munich, Germany

Ralf Dittrich University Hospital Erlangen, OB/GYN, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany

Tanja Fehm Department of Gynaecology & Obstetrics, Heinrich-Heine University Düsseldorf (HHU), Düsseldorf, Germany

Martin F. Fey University Clinic for Medical Oncology, University of Bern, Bern, Switzerland

Ariane Germeyer Department of Gynaecological Endocrinology and Fertility Disorders, University Women's Hospital Heidelberg, Heidelberg, Germany

Maren Goeckenjan TU Dresden, University Hospital, Dresden, Germany

Nicola Gökbuget Department of Medicine II, Haematology/Oncology, Goethe University, University Hospital, Frankfurt, Germany

Joerg Henes Department of Internal Medicine II (Haematology, Oncology, Immunology and Rheumatology), University Hospital Tuebingen, Tuebingen, Germany

Melanie Henes Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, Tuebingen, Germany

Jens Hirchenhain UniKiD, University Centre for Assisted Reproductive Medicine, University Hospital Duesseldorf, Duesseldorf, Germany

Sara Imboden Department of Obstetrics and Gynecology, University Women's Hospital, University of Bern, Bern, Switzerland

Andrea Jarisch Division of Stem Cell Transplantation and Immunology, Department for Children and Adolescents, University Hospital, Goethe University, Frankfurt am Main, Germany

Sabine Kliesch Department of Clinical and Surgical Andrology, Centre of Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany

Matthias Korell Department of Gynaecology and Obstetrics, Johanna Etienne Hospital, Neuss, Germany

Jana Liebenthron UniCareD, University Cryobank for Assisted Reproductive Medicine and Fertility Protection at UniKiD Duesseldorf, University Hospital Duesseldorf, Duesseldorf, Germany

Frank Nawroth Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology, amedes MVZ Hamburg, Hamburg, Germany

Kenny A. Rodriguez-Wallberg Department of Reproductive Medicine and Oncology-Pathology, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden

Patrizia Sager Breast Centre Bern Biel, Hirslanden Campus Bern, Bern, Switzerland

Nicole Sänger Department of Gynaecological Endocrinology and Reproductive Medicine, University Hospital Bonn, Bonn, Germany

Andreas Schüring MVZ KITZ Fertility Centre, Regensburg, Germany

Alexandra S. Kohl Schwartz Division of Gynecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland

Petra Stute Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland

Moritz Suerdieck Gyn-A.R.T. AG, Zürich, Switzerland

Pauline Wimberger TU Dresden, University Hospital, Dresden, Germany

Michael von Wolff Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland

Department of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland

Volker Ziller Dep. Gyn. Endocrinology and Reproductive Medicine, Clinic for Gynaecology and Obstetrics, University Hospital Giessen and Marburg (UKGM), Marburg, Germany

Abbreviations

ABVD	Adriamycin, Bleomycin, Vinblastine, Dacarbazine
AEH	Atypical endometrial hyperplasia
AFC	Antral follicle count
AGO e.V.	German Gynaecological Oncology Working Group
ALD	X-linked adrenoleukodystrophy
ALL	Acute lymphoblastic leukemia
AMH	Anti-Mullerian hormone
AML	Acute myeloid leukemia
ANCA	Anti-neutrophil cytoplasmic antibodies
APS	Antiphospholipid antibody syndrome
ARA-C	Cytarabine
ART	Assisted reproductive technologies
ASCO	American Society of Clinical Oncology
ASRM	American Society for Reproductive Medicine
AWMF	*Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften* (Working Group of the Scientific Medical Societies)
BEACOPP	Bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone
BOT	Borderline ovarian tumor
BRCA	Breast cancer
CDA	Congenital dyserythropoietic anemia
CEP	Congenital erythropoietic porphyria
CGD	Chronic granulomatous disease
CI	Confidence interval
CIP	Ceramide-I phosphate
CMA	Chlormadinone acetate
CML	Chronic myeloid leukemia
CNS	Central nervous system
COC	Combined oral contraceptives
CYC	Cyclophosphamide

DGGG	German Society of Gynaecology and Obstetrics
D.I.R.	German IVF registry
DVR	Umbrella Organisation for Reproductive Biology and Reproductive Medicine
EBMT	European Society for Blood and Marrow Transplantation
ECG	Electrocardiogram
EFI	Endometriosis fertility index
EC	Endometrium carcinoma
ECG	Electrocardiogram
EMA	European Medicines Agency
ESHRE	European Society of Human Reproduction and Embryology
ESMO	European Society for Medical Oncology
EULAR	European League Against Rheumatism
FFTF	Freedom from treatment failure
FIGO	International Federation of Gynecology and Obstetrics
FSH	Follicle-stimulating hormone
FtM	Female to male
GAHT	Gender-affirming hormone treatment
GHSG	German Hodgkin Study Group
GnRH	Gonadotropin-releasing hormone
GnRHa	Gonadotropin-releasing hormone agonists
GnRHant	Gonadotropin-releasing hormone antagonists
GPOH	German Society for Paediatric Oncology and Haematology
Gy	Gray
HCG	Human chorionic gonadotropin
HL	Hodgkin's lymphoma
HLH	Hemophagocytic lymphohistiocytosis
HMG	Human menopausal gonadotropin
HNPCC	Hereditary nonpolyposis colorectal carcinoma
HPV	Human papilloma virus
HRT	Hormone replacement therapy
HSCT	Hematopoietic stem cell transplantation
ICSI	Intracytoplasmic sperm injection
IL	Interleukin
ISFP	International Society for Fertility Preservation
IUD	Intrauterine device
IUI	Intrauterine insemination
IVF	In vitro fertilization
IvG	In vitro growth
IVM	In vitro maturation
LAD	Leukocyte adhesion deficiency syndrome
LH	Luteinizing hormone

LLETZ	Large loop excision of the transformation zone
MDS	Myelodysplastic syndrome
MESA	Microsurgical epididymal sperm aspiration
MLD	Metachromatic leukodystrophy
MPA	Medroxyprogesterone acetate
MRD	Minimal residual disease
MRI	Magnetic resonance imagine
MRS	Menopause rating scale
MtF	Male to female
NC	Natural cycle
NETA	Norethisterone acetate
NHL	Non-Hodgkin's lymphoma
OEGGG	Austrian Society of Gynaecology and Obstetrics
OS	Overall survival
PCOS	Polycystic ovary syndrome
PDWP	Paediatric Diseases Working Party
PFS	Progression-free survival
PGT	Preimplantation genetic testing
POI	Premature ovarian insufficiency
POP	Progestogen-only pill
PPOS	Progesterone-primed ovarian stimulation
RC	Refractory cytopenia
SAA	Severe aplastic anemia
SCD	Sickle cell disease
SCID	Severe combined immunodeficiency
SGGG	Swiss Society of Gynaecology and Obstetrics
SLE	Systemic lupus erythematosus
SNRI	Selective serotonin and norepinephrine reuptake inhibitors
SOP	Standard operation procedure
SRS	Sex reassignment surgery
SSC	Spermatogonial stem cells
SSRI	Selective serotonin reuptake inhibitors
TBI	Total body irradiation
TESE	Testicular sperm extraction
TMMR	Total mesometrial resection
TRT	Tissue hormone replacement therapy
TOS	Therapy optimization studies
TS	Turner syndrome
UAE	Uterine artery embolization
VTE	Venous thromboembolism
WHO	World Health Organization
WPATH	World Professional Association for Transgender Health

Part I
Before Fertility Preservation

How to Use the Book

Michael von Wolff and Frank Nawroth

The structure of this book is based on the course of everyday clinical practice. The following questions must be discussed in everyday clinical practice when a patient attends the clinic:

1. Is there an indication for fertility-preservation treatment?
2. How do I define the indication for fertility-preservation treatment?
3. What measures can be taken, how, with what effectiveness and which risks?

The answer to the first question is essentially found in Part I in the chapter "Indications for and Against Fertility Preservation", the answer to the second question is in the chapters of Part II and the answer to the third question is in the chapters of Part III.

Part I (see chapter "Indications for and Against Fertility Preservation") gives an overview of indications and presents the necessary criteria for defining an indication (Fig. 2).

Part II deals predominantly with common diseases. Less common malignancies are incorporated into chapter "Other Malignancies". The chapters were largely structured in such a way that first the disease prognosis, then the probability of damage to the gonads by the expected treatment, followed by the disease-specific risks of fertility-preservation treatment are presented. They end with the practical

M. von Wolff (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

F. Nawroth
Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology, amedes MVZ Hamburg, Hamburg, Germany
e-mail: Frank.Nawroth@amedes-group.com

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_1

approach outlined in a flow chart. The general gonadotoxic risks of radiotherapy are described in the chapter "Indications for and Against Fertility Preservation".

Part III explains the established or largely established fertility-preservation techniques. Where appropriate, the rationale, effectiveness and risks of each technique are presented. Information on a practical approach is available at the end. Less established techniques are briefly presented in the chapter "Further Fertility Preservation Techniques".

The following question may arise during the further course of patient care:

1. *What is the procedure after fertility-preservation treatment?*
   In Part IV, we have therefore added frequent and clinically relevant topics such as the treatment of uterine bleeding during chemotherapy, infertility therapy after fertility preservation therapies, pregnancy after chemotherapy and radiation of the pelvis and what to do if premature ovarian insufficiency occurs.
2. *How do I justify the reimbursement of costs for fertility-preservation treatment to the health payer?*
   In some countries, both the indication and the choice of fertility-preservation treatments must be justified to the health insurance company or another institution. The data for such justification will be provided in the book as far as possible. It is therefore advisable to name and reference the relevant chapters.

Further Information

National and International Guidelines

- *German Austrian Swiss S2k AWMF Guidelines* (*A*rbeitsgemeinschaft der *W*issenschaftlichen *M*edizinischen *F*achgesellschaften)
  German full version: https://www.awmf.org/uploads/tx_szleitlinien/015-082l_S2k_Fertilitaetserhaltung-bei-onkologischen-Therapien_2017-12.pdf.
  English short version (PubMed open access): Dittrich R, Schuering A, Kiesch S et al. Fertility preservation for patients with malignant disease. Guideline of the DGGG, DGU and DGRM (S2k-Level, AWMF Registry No. 015/082, November 2017) – recommendations and statements for girls and women. Geburtshilfe Frauenheilkd 2018;78:567–84.
- *ASCO guideline 2018* (American Society of Clinical Oncology): Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, Wallace WH, Wang ET, Loren AW. Fertility preservation in patients with cancer: ASCO Clinical Practice Guideline Update. J Clin Oncol 2018;36:1994–2001.
- *ESHRE-Guideline 2020* (European Society of Human Reproduction and Embryology) (to be published in 2020).
- *ESMO-Guideline 2020* (European Society for Medical Oncology) (to be published in 2020).

*Ferti*PROTEKT *Recommendations 2018 (PubMed Open Access)*

- *Part I*: Schüring AN, Fehm T, Behringer K, Goeckenjan M, Wimberger P, Henes M, Henes J, Fey MF, von Wolff M. Practical recommendations for fertility preservation in women by the *Ferti*PROTEKT network. Part I: Indications for fertility preservation. Arch Gynecol Obstet 2018;297:241–55.
- *Part II*: von Wolff M, Germeyer A, Liebenthron J, Korell M, Nawroth F. Practical recommendations for fertility preservation in women by the *Ferti*PROTEKT network. Part II: fertility preservation techniques. Arch Gynecol Obstet 2018;297:257–67.

*Information About the Ferti*PROTEKT *Network (PubMed Open Access)*

- von Wolff M, Andersen CY, Woodruff TK, Nawroth F. Oncofertility Consortium and the Danish Fertility-Preservation Networks—what can we learn from their experiences? Clin Med Insights Reprod Health 2019;13:1179558119845865.

Information for Patients (English and German)

- *Website FertiPROTEKT*: www.fertiprotekt.com; www.fertiprotekt.de; www.fertiprotekt.ch; www.fertiprotekt.at

Networks for Fertility Preservation

Frank Nawroth and Michael von Wolff

Fertility protection requires close coordination of reproductive physicians, reproductive biologists and oncologists from a variety of disciplines. Because of these characteristics, it is imperative that all scientific and clinical areas involved organize themselves into a network structure both as a medical–logistic network and as a medical professional society.

The necessary network structures can vary considerably at regional, national and international level, since the size of the regions to be integrated, the local cultural and geographical conditions and the political conditions vary greatly. In addition to *Ferti*PROTEKT network, which operates both centrally and decentrally, there are other important network structures whose structure depends especially on the size of the region to be covered.

- The Danish network (www.rigshospitalet.dk) is a typical example of a smaller country. It is centralized, excellently serving the practical implementation of specific fertility-preserving techniques such as cryopreservation and transplantation of ovarian tissue, enabling high-quality scientific studies and a detailed data registry.
- The German-speaking three-country network *Ferti*PROTEKT® (www.fertiprotekt.com) is a typical example of a larger country. It is both centralized and decentralized, enabling scientific studies and a data registry with a high number of cases.

F. Nawroth (✉)
Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology, amedes MVZ Hamburg, Hamburg, Germany
e-mail: Frank.Nawroth@amedes-group.com

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_2

- The Oncofertility® Consortium (www.oncofertility.northwestern.edu) operates globally and is a decentralized network that primarily serves the transfer of knowledge among its members.

Networks are often modular, with the number of modules depending on the size of the network (Fig. 1). The conditions and intentions of these modules are different [1].

The smallest modular unit usually forms a reproductive medical centre or clinic, which networks with the oncologists regionally or internally. Patients are assigned to the reproductive medicine centres directly by the oncologists. The treatment decision is often based on direct bilateral communication. The reproductive medicine centre documents the treatments so that data can later be passed on to a registry.

The next medium-size modular stage is a union of local units into a small national or large regional network. An example of such a network is Denmark (www.rigshospitalet.dk). The centres know each other, which facilitates unproblematic personal communication. Data from the local units is merged into a register, which is relatively easy to create due to its limited size. It is possible to establish centralized, highly specialized facilities, e.g. for cryopreservation of gonadal tissue. The establishment of such centralized facilities allows high-quality fertility-protective techniques, scientific evaluation, good transparency of activities and thus healthcare policy initiatives. Due to short travel distances, briefer training courses can be organized with the involvement of oncologists. The strengths of these medium-sized

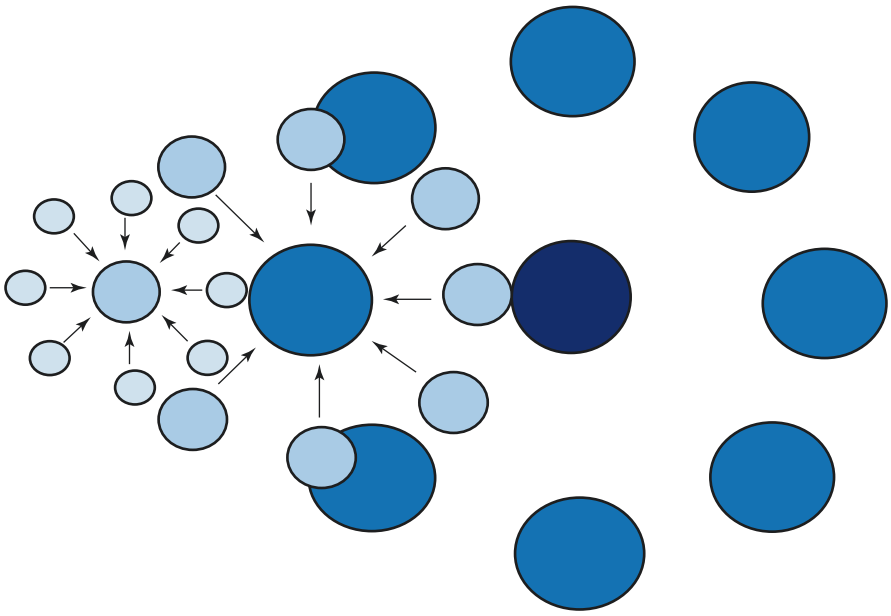


Fig. 1 Modular structure of networks (modified after [1])

networks lie in the ability of being able to collect high-quality data, since usually extremely detailed data documentation is possible.

In networks of large regions or in larger countries, several groups, such as in Denmark, are combined into a large network. One such example is the *Ferti*PROTEKT® network.

It is also possible to combine several of these networks for data collection and professional exchange. Examples of such international networks are the Oncofertility® Consortium (www.oncofertility.northwestern.edu), the ESHRE Special Interest Group, "Fertility Preservation" (www.eshre.eu/Specialty-groups/Special-Interest-Groups/Fertility-Preservation.aspx) and the "International Society for Fertility Preservation", ISFP (www.isfp-fertility.org).

Reference

1. von Wolff M, Yding Andersen C, Woodruff T, Nawroth F. *Ferti*PROTEKT, Oncofertility consortium and the Danish fertility preservation networks—what can we learn from their experiences? Clin Med Insights Reprod Health. 2019;13:1179558119845865. https://doi.org/10.1177/1179558119845865.

*Ferti*PROTEKT Network

Frank Nawroth, Moritz Suerdieck, and Michael von Wolff

Although scientific studies on fertility protection already existed in Germany at the beginning of the millennium, no concerted counselling and care for the patients had yet been established. In May 2006, at the initiative and invitation of Prof. M. von Wolff (then Department of Gynaecological Endocrinology and Fertility Disorders in Heidelberg) and Prof. M. Montag (then Department of Gynaecological Endocrinology and Reproductive Medicine in Bonn), 30 university reproductive medical centres met in Heidelberg for the foundation of the network *Ferti*PROTEKT, in which private centres can also become members since 2008.

Among other things, the network should meet the following objectives:

- Creation of comprehensive structures for the implementation of consultation/counselling and fertility treatment therapies
- Professional content and interdisciplinary coordination of consultation/counselling and therapies for fertility protection
- Documentation of consultation/counselling and therapies in a register
- Initiation, implementation and support of studies
- Definition of standards and publication of recommendations

F. Nawroth (✉)
Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology, amedes MVZ Hamburg, Hamburg, Germany
e-mail: Frank.Nawroth@amedes-group.com

M. Suerdieck
Gyn-A.R.T. AG, Zürich, Switzerland
e-mail: moritz@suerdieck.com

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_3

Despite its national and international high profile, e.g. in cooperation with other professional societies, in the preparation of guidelines, discussions with health insurance companies, etc. the network did not have a recognized legal form. It was therefore converted into a registered association and the *Ferti*PROTEKT network was founded on November 10, 2015 in Hamburg. Its head office is in Marburg, Germany.

Since the end of 2017, the association has been a member of the "Umbrella organisation Reproductive Biology and Reproductive Medicine" (DVR), and a *Ferti*PROTEKT network meeting also takes place at DVR conferences (every 2 years). Therefore, in 2018, it was decided that the annual working meetings would only be held every 2 years, alternating with the DVR congress. Since the end of 2018, the *Ferti*PROTEKT network has also been a cooperating society of the German Society for Gynaecology and Obstetrics (DGGG).

The association has 143 member centres (Germany: 124, Austria: 12, Switzerland: 7, as of May 2020) (Fig. 1). With the application for admission, each centre must prove that it can provide fertility-protective measures on its own or, if necessary, in a specifically named cooperation.

The documentation of all consultations and therapies in a separate register until the end of 2017 has been supported by a cooperation with the German IVF registry (D.I.R.) since 2018. Figure 2 shows the consultations carried out until 2018 and the resulting fertility-protective interventions.

More than a decade of intensive work by the respective management teams/boards, combined with the exemplary joint efforts of all member centres has resulted in increasingly comprehensive consultation and treatment options, local logistical networks for optimal patient care, intensive exchange between centres and numerous study activities and publications.

In more than 10 years, a consolidation of several centres interested in the protection of fertility (especially, but not exclusively oncology patients) has developed into a registered association with well over 100 member centres from three countries and created the basis for the widest possible provision of consultation and treatment options. Scientific studies have been initiated and/or made possible, and recommendations and standards have been agreed (Fig. 3).

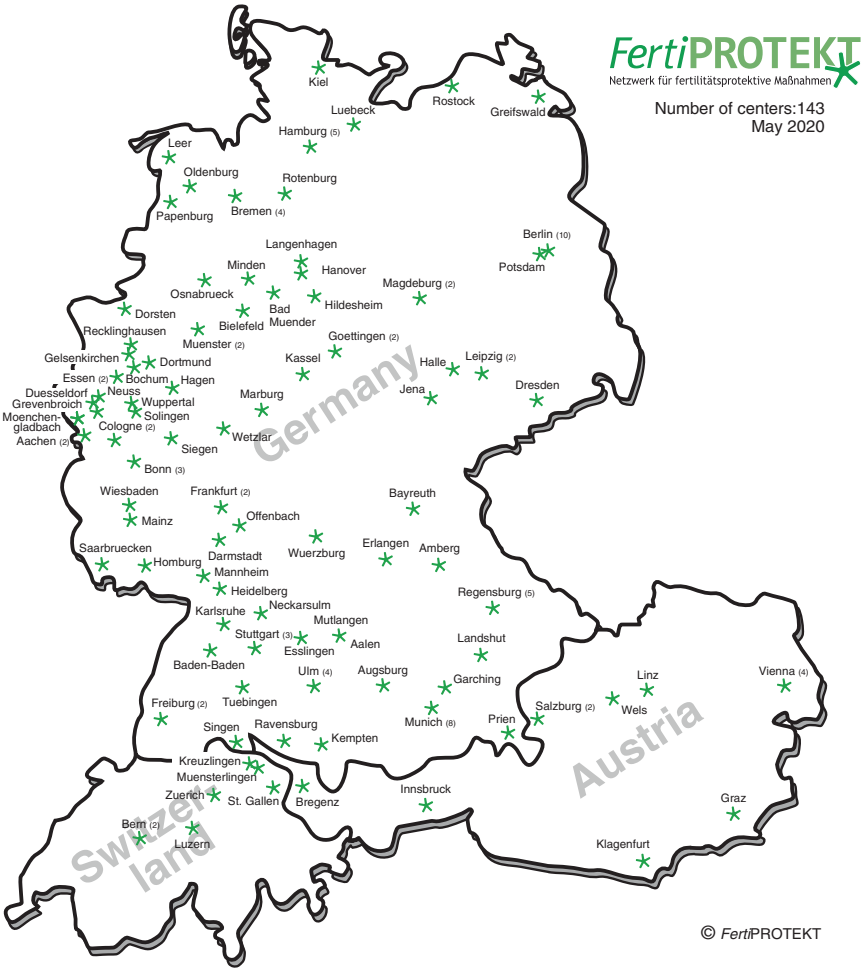


Fig. 1 Graphic presentation of the 143 member centres (May 2020)

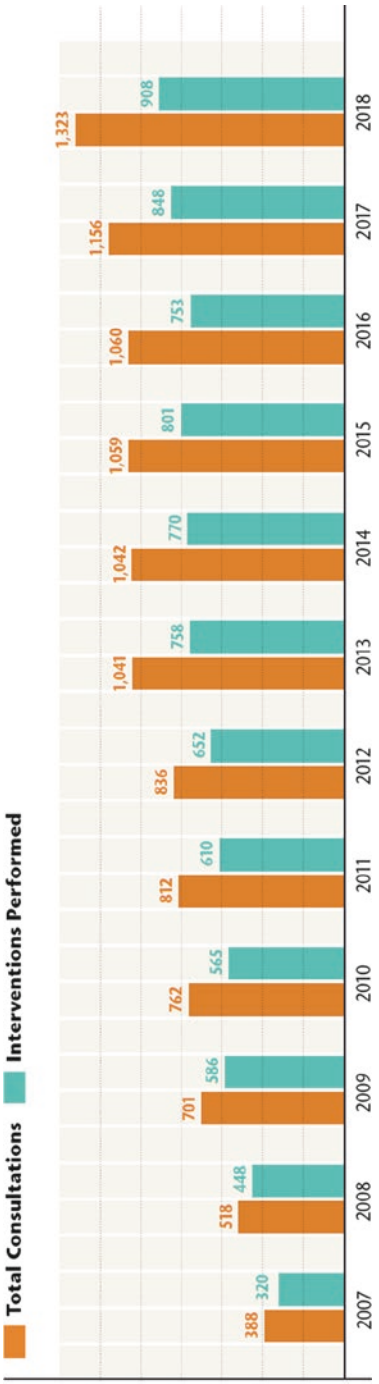


Fig. 2 Total consultations and interventions performed in the *Ferti*PROTEKT network between 2007 and 2018 [1]

Fig. 3 Development of *Ferti*PROTEKT network

Reference

1. Germeyer A, Dittrich R, Liebenthron J, Nawroth F, Sänger N, Suerdieck M, von Wolff M. D.I.R. annual report 2018. J Reprod Med Endocrinol. 2019;16:41–4.

Logistics for Fertility-Preservation Counselling

Anke Barnbrock and Nicole Sänger

Introduction

Preserving fertility before gonadotoxic treatment in adults has become increasingly important in recent years because of improved prognoses and survival rates, which allow later pregnancy and motherhood. In addition to the acceptance of colleagues to provide information on this topic, the clarification on and implementation of fertility preservation measures have now been incorporated into current guidelines [1]. With the enactment of the Appointment Service and Care Act in May 2019, the path to the nationwide adoption of fertility preservation measures by health insurance funds is also mapped out in Germany [2], as is already common practice in many other countries. This will mean a drastic increase in requests for fertility preservation measures in oncological and reproductive medicine departments and this topic will increasingly become a focus of attention for doctors and affected families as well as for pre- and post-puberty children and adolescents. Fertility counselling for patients, which is adapted to the gonadotoxic risk and takes individual factors into account, and which should also be offered comprehensively and in a structured manner in oncology centres for all patients, is still currently a major challenge.

A. Barnbrock (✉)
Clinic for Paediatric and Adolescent Medicine, Department of Oncology, Haematology and Haemostaseology, University Hospital Frankfurt/Main, Goethe-University Frankfurt, Frankfurt, Germany
e-mail: Anke.Barnbrock@kgu.de

N. Sänger
Department of Gynaecological Endocrinology and Reproductive Medicine, University Hospital Bonn, Bonn, Germany
e-mail: Nicole.Saenger@ukbonn.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_4

Multidisciplinary Team

A first step and basic prerequisite for establishing fertility counselling in oncological centres is the establishment of contacts and networking with specialist disciplines involved in the care of patients undergoing a potentially gonadotoxic treatment. A prerequisite for the integration of the topic of fertility and fertility maintenance in connection with oncological diseases is initially raising awareness and the associated willingness to deal with this topic for patients. The *Ferti*PROTEKT network [3], as well as the German Childhood Cancer Foundation [4], provides online information material to sensitise colleagues and patients about this topic and to enable networking with corresponding centres.

Fertility counselling prior to planned gonadotoxic treatment is only useful if there is sufficient time to carry out potentially fertility-preserving measures. Advice on alternative options if there is too little time available before the start of treatment and on endocrinological aftercare should be offered to all patients by means of reproductive medical counselling, even after completion of the gonadotoxic treatment. Therefore, it is an important prerequisite that all specialist disciplines involved work closely together (Fig. 1).

In addition to the supervising oncologists or paediatric oncologists, transplant surgeons and gynaecologists, the team also includes reproductive physicians. Experienced gynaecologists and paediatric surgeons are required to perform invasive surgical measures (ovarian tissue cryopreservation, ovarian transposition). Radiologists should also be involved in the assessment and indication of ovarian

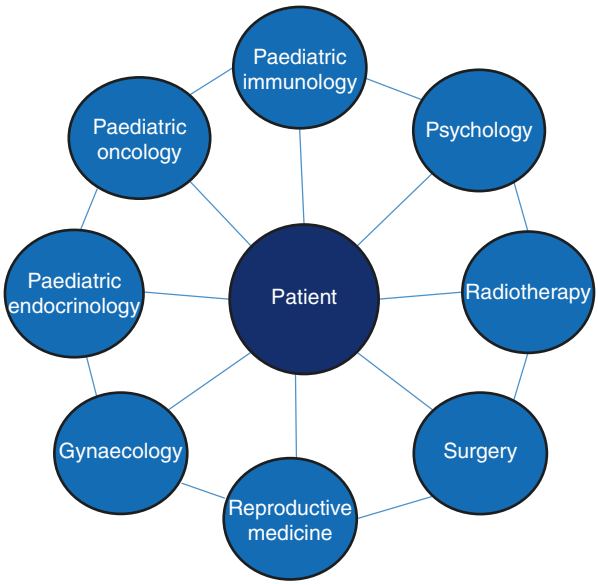


Fig. 1 Multidisciplinary team for optimal fertility consulting in paediatrics

transposition to move the ovary away from the radiation field in patients undergoing radiotherapy. Close cooperation with gynaecological/paediatric endocrinologists is essential, e.g. for clarifying the pubertal stage in advance and for patient follow-up. As the subject of fertility and the consequences are very sensitive issues for patients, it is also helpful to involve psychologists in the team who also accompany the counselling processes. It is beneficial to designate a fertility advisor (also known as an "oncofertility navigator"), who initiates and coordinates advice and the networking of all disciplines (see also Fig. 3). Such a role is certainly optional, but has proven to be helpful for the smooth running of counselling and the initiation of procedures. All available disciplines should be prepared to make appointments at short notice for consultation, presentation or even the implementation of invasive measures, since—due to the basic diagnosis—the time available for fertility preservation treatment can be tight and a rapid start of treatment should not be delayed by the fertility consultation. Particularly with paediatric patients, an appropriate approach to their maturity is a matter of course and necessary during their counselling and support.

Consensus Agreement Amongst the Team

The prerequisite for fertility counselling of oncological patients prior to gonadotoxic treatment or stem cell transplantation is firstly the appreciation and recognition of this topic among the doctors caring for them and secondly a unified policy in the counselling of patients and the indications for fertility preservation measures. The team should take note of and discuss existing recommendations on fertility preservation, for example from the German Society for Gynaecology and Obstetrics [5], the Society for Paediatric Haematology and Oncology [6] or the European Stem Cell Society (EBMT) [7] (Fig. 2).

The existing guidelines help to classify the gonadotoxic risk of the respective treatment and indicate possibilities for fertility preservation. When advising patients, however, other individual aspects must be considered. The consensus among colleagues is important here to also enable a standard procedure for the patients and factors such as the time delay or the patient's general state of health. Despite the importance of informing patients before the start of treatment and the significance of fertility for the patients' future, fertility counselling and possibly associated measures for preserving fertility should not lead to a relevant postponement of the start of treatment or to surgical complications, to a worsening of the patient's prognosis.

An important point in the decision for or against fertility preservation measures is to clarify the risk of transmission of malignant cells through gonadal tissue. The respective risk should be considered carefully and openly discussed with the patient when deciding whether to remove gonadal tissue. Further aspects to be considered here are the curative or palliative orientation of the treatment, the maturity and pubertal development of paediatric patients, as well as the localisation of a tumour and the associated surgical complications if invasive measures are performed. If there are underlying genetic diseases, which can be passed on to the offspring if the

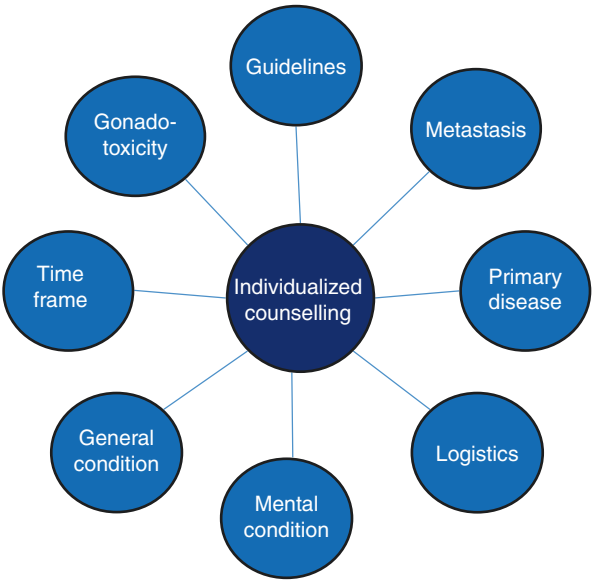


Fig. 2 Influencing factors for individual fertility counselling

tissue is preserved, corresponding education of the patient or their legal guardian as well as human genetic counselling after the end of the treatment is necessary.

Ethical issues must also be discussed in the team, since, for example, the legal guardians of paediatric patients must decide about using tissue from their underage child long before the subject of family planning can be considered.

Structures

Standard Operating Procedures (SOPs)

To enable comprehensive and prompt fertility counselling in oncology centres, it is helpful to structure necessary procedures and tasks.

If the centre is a practice or clinic without its own oncology department, it is advisable to look for cooperation partners and organise a standard procedure. Cooperation agreements are necessary for this. Fixed contact persons, easily accessible telephone contacts and standardised transport (e.g. for tissue) also facilitate rapid consultation and treatment of the patients concerned, especially when there is time pressure.

Overall, it makes sense to determine which parameters should be processed before the start and during the course of a potentially gonadotoxic treatment and to document the necessary examinations and procedures required, e.g. in the checklists when patients are newly admitted to oncology departments or stem cell

transplant centres. Standard operating procedures (SOP) can be helpful in structuring the procedures for fertility counselling and the implementation of fertility-preservation measures. There are therefore fixed pathways for patients to receive all the necessary preliminary investigations and to quickly receive fertility counselling as part of the staging of new diagnoses (Fig. 3).

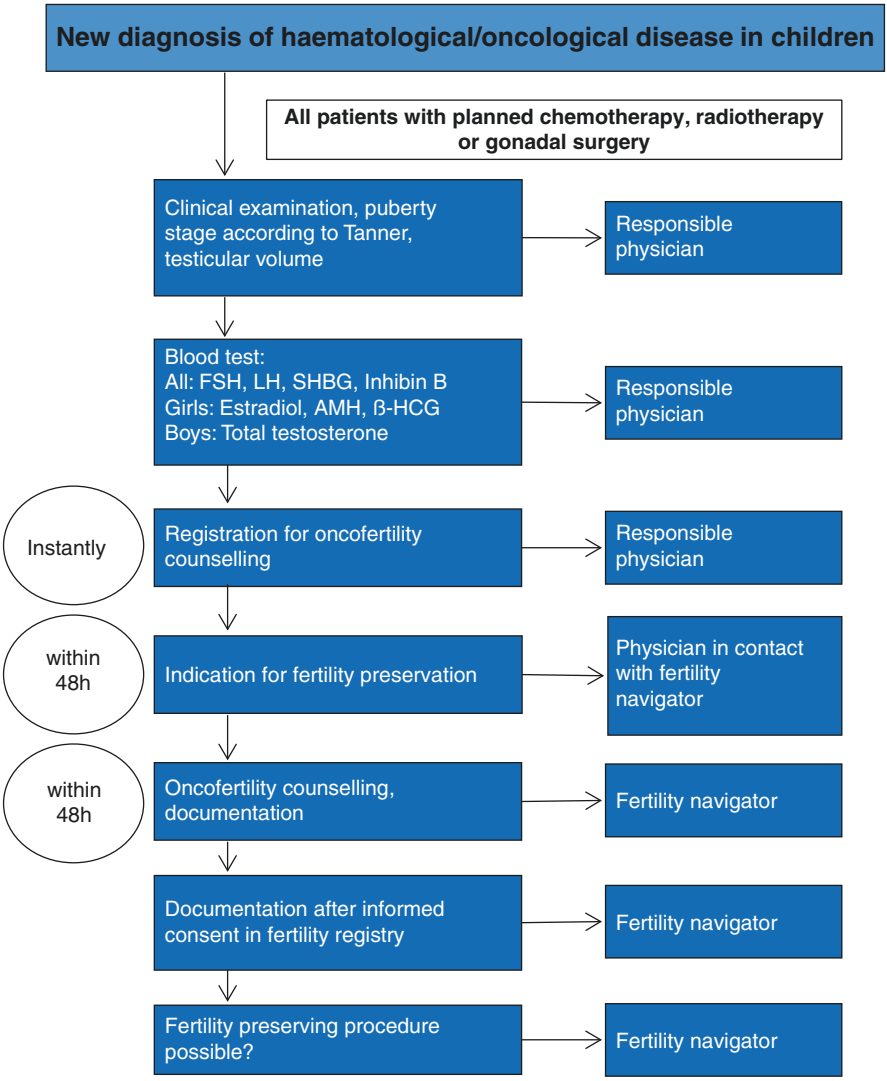


Fig. 3 Example of a standard operating procedure (SOP) for fertility counselling of paediatric patients. The oncofertility navigator/fertility consultant advises the patient and initiates the implementation of fertility-preservation measures

Investigation

In addition to the medical history, a gynaecological examination and ultrasound of the internal genitals should be performed as part of fertility-preservation planning. In the consultation and care of paediatric patients, it is also necessary to determine the puberty stage prior to consultation, since—depending on maturity and development—different fertility preservation methods may be considered.

These include:

- Asking about the menarche
- Clinical examination
- Documentation of the Tanner stage
- Measurement of weight and height
- Measurement of FSH, LH, estrogen and AMH

Depending on the legal situation in the respective country, infection serology (HIV, hepatitis B and C) is also required before the storage of germ cells or tissue to avoid potential transmission of contaminants to already stored samples [8]. Separate cryopreservation and storage are necessary for infectious patients.

Legal Aspects of Removal and Storage

In order to be able to store cryopreserved fertilised or unfertilised oocytes, expert preparation and treatment by a specialist in reproductive medicine are required. The existence of an IVF laboratory and the expertise of embryologists are considered a basic requirement for fertility preservation (as well as in assisted reproduction in event of an unfulfilled desire to have children) to adequately process the germ cells according to scientific standards. The quality of further treatment plays a decisive role for the later outcome in terms of the live birth rate.

Reproductive medicine centres and their IVF laboratories are subject to legal regulations, which are checked by regular inspections. In the case of storage or relocation of externally removed germ cells, the above-mentioned cooperation agreements and SOPs must be kept in place and regulated transport must be organised to guarantee a standard and legally compliant therapy [9, 10]. It is therefore advisable to devote time and attention to recommendations/guidelines and the legal situation relating to reproductive medicine therapy, removal and storage of germ cells or tissue (e.g. removal and transfer of human germ cells as part of assisted reproduction, the Medicines Act, transplantation law, ESHRE guidelines) [11, 12].

In the case of cryopreservation of ovarian tissue—similar to the removal of oocytes—depending on the country, permission from the government authorities may be required for the inspection and release of the surgeons and the operating theatres [8]. A prerequisite for this is approval from the respective regional council before the tissue can be removed and transferred to a specialised centre with a

cryobank. This applies not only to gynaecological but also to paediatric surgeons, who are required to provide evidence of an appropriately trained and qualified person. There does not seem to be a nationwide or even international standard regulation for this, and it is at the discretion of the individual regional councils.

The processing of ovarian tissue prior to cryopreservation, its transport, as well as the freezing and thawing process are different from that of cryopreservation of germ cells and should be subject to qualitative standards, which is why cooperation with specialized centres is recommended.

Before oocytes or ovarian tissue are removed for fertility preservation purposes, the following should be contractually clarified in addition to obtaining written consent from the patient or legal guardian: how long the material will be kept frozen for, at what intervals the patient will request renewal of his consent for storage and that the material will be destroyed in the event of the patient's death [13, 14].

Aftercare

After completing gonadotoxic treatment, regular long-term follow-up is recommended to resolve endocrinological problems e.g. the possibly necessary induction of puberty or gynaecological questions e.g. to discuss sexuality, fertility, contraception or hormone replacement therapy (Chapters "Infertility Treatment After Fertility Preservation Therapies", "Pregnancy After Chemotherapy and Radiation of the Pelvis", and "Premature Ovarian Insufficiency: Hormone Replacement Therapy and Follow-up"). An individual assessment of the expected loss of ovarian function must take various factors into account:

- The age of the patient
- The therapy regime
- Other pre-existing conditions
- The existing ovarian reserve

The latter is only possible to a limited extent, especially in childhood and adolescence, since the determination of the concentration of Anti-Müllerian hormone (AMH) and the ultrasound representation of the antral follicle count (AFC) only allow approximate conclusions to be drawn about the primordial follicles which are actually present.

Documentation

Documentation forms are useful to ensure that the fertility counselling carried out and indicated fertility preservation measures are well documented at a later date. Analogous to classic ART patients and in accordance with legal requirements, they must be stored for 30 years. A summary of the consultation in the respective oncological epicrisis is recommended and is considered legal documentation of a

completed explanation of the expected gonadotoxic risk of the planned treatment. If fertility-preserving measures have been carried out, an additional doctor's letter ("tissue passport") may be useful, which contains information about the consultation, measures carried out and complications, and in particular the transport and whereabouts of the tissue (location of the tissue bank), to ensure that patients can retrieve the tissue later if necessary.

References

1. Dittrich R, Kliesch S, Schüring A, Balcerek M, Baston-Büst D, Beck R, et al. Fertility preservation for patients with malignant disease. Guideline of the DGGG, DGU and DGRM (S2k-Level, AWMF Registry No. 015/082, November 2017)—recommendations and statements for girls and women. Geburtshilfe Frauenheilkd. 2018;78:567–84.
2. https://www.bgbl.de/xaver/bgbl/start.xav?startbk=Bundesanzeiger_BGBl&start=%2F%2F%2A%5B%40attr_id=%27bgbl119s0646.pdf%27%5D#__bgbl__%2F%2F*%5B%40attr_id%3D%27bgbl119s0646.pdf%27%5D__1558450984880
3. www.fertiprotekt.com
4. www.kinderkrebsstiftung.de
5. www.dggg.de
6. www.gpoh.de
7. Dalle JH, Lucchini G, Balduzzi A, Ifversen M, Jahnukainen K, Macklon KT, et al. State-of-the-art fertility preservation in children and adolescents undergoing haematopoietic stem cell transplantation: a report on the expert meeting of the Paediatric Diseases Working Party (PDWP) of the European Society for Blood and Marrow Transplantation (EBMT) in Baden, Austria, 29-30 September 2015. Bone Marrow Transplant. 2017;52:1029–35.
8. https://www.gesetze-im-internet.de/amg_1976/__20b.html
9. https://www.bundesaerztekammer.de/richtlinien/richtlinien/assistierte-reproduktion/
10. https://www.gesetze-im-internet.de/tpg/TPG.pdf
11. ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine. The Vienna consensus: report of an expert meeting of ART performance indicators. Reprod Biomed Online. 2017;35:494–510.
12. ESHRE Guideline Group on Good Practice in IVF Labs. Revised guidelines for good practice in IVF laboratories (2015). Hum Reprod. 2016;31:685–6.
13. Beckmann MW, Lotz L, Toth B, Baston-Büst DM, Fehm T, Frambach T, et al. Concept paper on the technique of cryopreservation, removal and transplantation of ovarian tissue for fertility preservation. Geburtshilfe Frauenheilkd. 2019;79:53–62.
14. Liebenthron J, Montag M, Reinsberg J, Köster M, Isachenko V, van der Ven K, et al. Overnight ovarian tissue transportation for centralized cryobanking: a feasible option. Reprod Biomed Online. 2019;38:740–9.

Indications for and Against Fertility Preservation

Michael von Wolff and Frank Nawroth

Basic Considerations

The criteria for performing fertility preservation are similar for men and women. However, since therapies for women are usually more complex, more expensive and are associated with higher risks, only the indications for women will be described below. Further information on the indications for fertility-preservation therapy in men can be found in the chapter "Cryopreservation of Sperm and Testicular Tissue" and for children in chapter "Pediatric Oncological Cancer".

If a woman is still of reproductive age, the first question that arises is whether fertility-preservation therapy should be offered or not. In principle, every woman should have the right to inform herself about the possibility of such measures. A study by the *Ferti*PROTEKT network showed that based on the (self-evaluated) physical and psychological status and the individual significance of fertility, it cannot be predicted whether a woman will decide for or against fertility-preservation therapy [1]. However, even those women who decided against fertility-preservation therapy found the counselling helpful.

Nevertheless, it does not make sense to send each patient to a specialist for fertility-preservation counselling, otherwise fertility-preservation measures could be taken even if they are not necessary because of low gonadotoxicity of the treatment. In addition, if the patient's prognosis is poor, unrealistic expectations and possibly unnecessary risks result from the implementation of such therapies.

M. von Wolff (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

F. Nawroth
Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology, amedes MVZ Hamburg, Hamburg, Germany
e-mail: Frank.Nawroth@amedes-group.com

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_5

Therefore, fertility-preservation therapy should only be used if:

- The likelihood of surviving the disease is high
- The risk of infertility due to (mostly) oncological therapy is high
- Fertility-preservation therapy is low risk and effective

Information on prognosis can be found in the disease descriptions in chapters "Breast Cancer; Hodgkin's Lymphoma; Acute Leukemia; Ovarian Tumors and Ovarian Cancer; Cervical Cancer; Endometrial Hyperplasia and Endometrial Carcinoma; Pediatric Oncological Cancer; Other Malignancies; Non-Malignant Diseases Requiring Stem Cell Transplantation; Severe Autoimmune Diseases; Endometriosis; Turner Syndrome; Transgender". Information on the risks of infertility due to oncological therapy is also described. These are highly dependent on the composition and dosage of the particular chemotherapy.

In this book, fertility-preservation therapy is recommended if (mostly) oncological therapy leads to a >20% probability of amenorrhea or azoospermia *at the time of the expected desire to have a child*. For example, the risk of amenorrhea immediately after chemotherapy does not play a role at the age of 18, but it does at the age of 30. The amenorrhoea/azoospermia threshold risk value of 20% is in line with the cost reimbursement policy in Switzerland. Fertility-preservation therapy costs are covered in Switzerland at a risk of >20%.

The specific risks of radiotherapy are shown in Table 1.

The so-called Edinburgh criteria are also helpful for defining the indication (Table 2). Wallace et al. [4] were able to demonstrate that if these criteria were met, ovarian tissue would only by cryopreserved if it was considered "useful" for the patient. A premature ovarian failure (POI) risk of >50% was regarded as "useful".

The girls and women who were offered ovarian tissue cryopreservation almost all survived the disease and had a 35% probability of premature ovarian failure. In comparison, the survival rate reached only 1% if such a therapy had not been offered according to the Edinburgh criteria.

Table 1 Effect of different radiation doses on the gonads [2, 3]

Effects	Radiation dose
Women: no relevant effects	0.6 Gy
Women: no relevant effects if age <40 years	1.5 Gy
Women: risk of ovarian insufficiency: ca. 60% if age 15–40 years	2.5–5 Gy
Women: sterilising dose at 0 years of age	20 Gy
Women: sterilising dose at 10 years of age	18 Gy
Women: sterilising dose at 20 years of age	16 Gy
Women: sterilising dose at 30 years of age	14 Gy
Women: sterilising dose at 40 years of age	7 Gy
Women: Reduction of the follicle pool by ca. 50%	2 Gy
Men: long-lasting azoospermia possible	≥2 Gy total
Men: permanent azoospermia possible	≥4 Gy total
Men: permanent azoospermia possible	≥1.2 Gy fractionated

Table 2 Edinburgh selection criteria for the cryopreservation of ovarian tissue in girls and young women [4]

Age <35 years
No prior chemotherapy if ≥15 years at first diagnosis
Mild, non-gonadotoxic chemotherapy acceptable if it occurred at the age of <15 years
Realistic chance of 5-year survival
High risk of premature ovarian failure (>50%)
No pregnancy and no own children

The type of fertility-preservation therapy to be chosen depends on many criteria such as the prognosis, the age of the patient, the available time frame, the risks of fertility-preservation therapy and ultimately also on the costs, the facilities at the counselling centres and the political situation. Due to the many factors involved, a decision on the type of therapy must always be made on an individual basis. The fertility-preservation interventions carried out in women and registered in the *Ferti*PROTEKT network in 2018 are shown in Fig. 1, depending on age.

Practical Approach

The following questions about the indication and implementation of fertility-preservation therapy arise in a logical sequence from the above explanations. The criteria mentioned must of course be adapted to the respective circumstances. Therefore, we deliberately refrain from quantifying the individual points, e.g. at exactly what risk of long-term sterility should fertility-preservation therapy be initiated, since the relevance varies greatly from individual to individual. Information on the questions can be found in chapters “Breast Cancer”; “Hodgkin’s Lymphoma”; “Acute Leukemia”; “Ovarian Tumors and Ovarian Cancer”; “Cervical Cancer”; “Endometrial Hyperplasia and Endometrial Carcinoma”; “Pediatric Oncological Cancer”; “Other Malignancies”; “Non-Malignant Diseases Requiring Stem Cell Transplantation”; “Severe Autoimmune Diseases”; “Endometriosis”; “Turner Syndrome”; and “Transgender”.

1. Does the patient’s age still allow the implementation of fertility-preservation therapy? If “yes”, i.e. if the patient is about ≤40 years old (in men there is no age limit), see Point 2.
2. Is the prognosis of the disease sufficiently good for the implementation of fertility-preservation therapy? If yes, see Point 3.
3. Is a later pregnancy (or paternity) compatible with the underlying disease and therapy? If “yes”, see Point 4.
4. Is the expected (mostly) oncological therapy associated with a relevant risk of long-term infertility? If “yes”, see Point 5.
5. Is it possible to carry out fertility-preservation therapy for the underlying disease with a low risk? If yes, i.e. if the health of the patient and the effectiveness of the oncological therapy are not endangered by the measures, see Point 6.

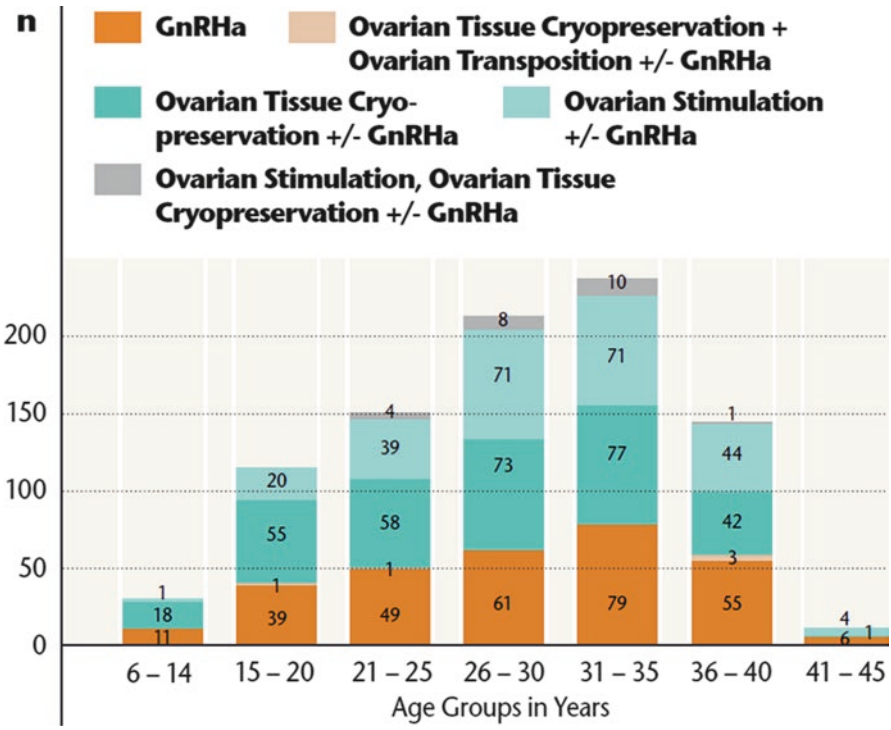


Fig. 1 Interventions carried out in 2018 among women in the *Ferti*PROTEKT network depending on age [5]

6. Is the time frame sufficient for carrying out fertility-preservation therapy? If yes, i.e. if ½—approx. 2–4 weeks (depending on the fertility-preservation measure) until the oncological therapy is initiated, see Point 7.
7. Which measure(s) should most reasonably be offered/carried out (see chapters "Ovarian Stimulation to Collect Oocytes"; "Cryopreservation of Unfertilized and Fertilized Oocytes"; "Removal of Ovarian Tissue"; "Transportation, Cryopreservation and Storage of Ovarian Tissue"; "Transplantation of Ovarian Tissue"; "GnRH Agonists"; "Transposition of Ovaries"; "Cryopreservation of Sperm and Testicular Tissue"; and "Further Fertility Preservation Techniques")?

Questions 1–6 are often already answered by the responsible oncologists. In individual cases, however, they are also discussed by the reproductive physicians before the patient is presented. If the questions tend to be answered with "yes", it is up to the reproductive physicians to discuss Question 7 with the patient and initiate appropriate measures. The flow chart (Fig. 2) shows the procedure diagrammatically.

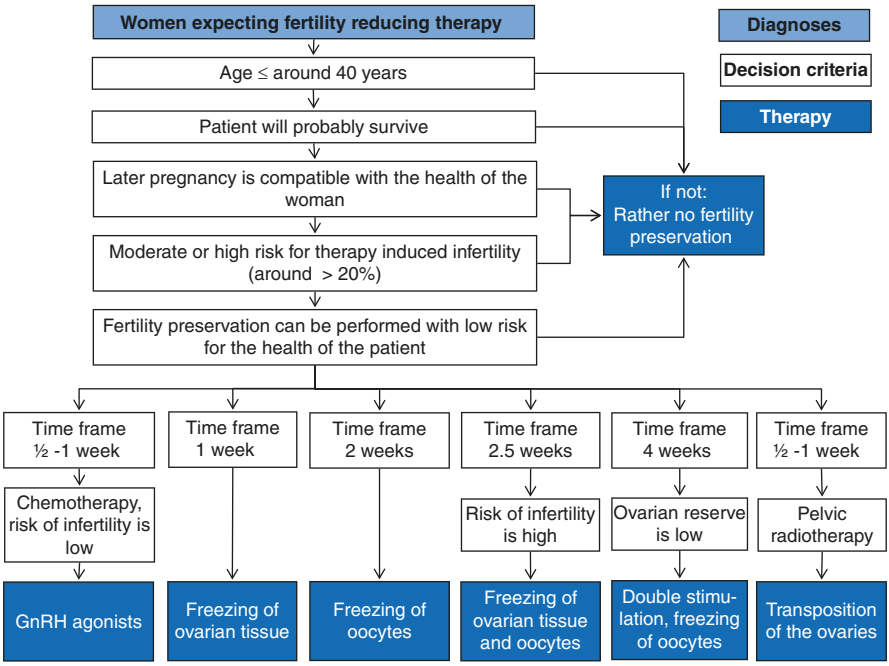


Fig. 2 Orientative approach for defining the indication for fertility-preservation therapy in women

References

1. von Wolff M, Giesecke D, Germeyer A, Lawrenz B, Henes M, Nawroth F, et al. Characteristics and attitudes of women in relation to chosen fertility preservation techniques - a prospective, multicenter questionnaire-based study. Eur J Obstet Gynecol. 2016;201:12–7.
2. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod. 2003;18:117–21.
3. Irtan S, Orbach D, Helfre S, Sarnacki S. Ovarian transposition in prepubescent and adolescent girls with cancer. Lancet Oncol. 2013;14:601–8.
4. Wallace WH, Smith AG, Kelsey TW, Edgar AE, Anderson RA. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. Lancet Oncol. 2014;15:1129–36.
5. Germeyer A, Dittrich R, Liebenthron J, Nawroth F, Sänger N, Suerdieck M, von Wolff M. D.I.R. annual report 2018. J Reprod Med Endocrinol. 2019;16:41–4.

Part II
Fertility Preservation—Diseases

Breast Cancer

Patrizia Sager, Michael von Wolff, and Tanja Fehm

Stage-Dependent Prognosis

Breast cancer is the most common cancer among women worldwide. In the Western world, the proportion of women under 40 years of age suffering from the disease is about 4–5%. This corresponds to around 3700 women per year in Germany and 280 per year in Switzerland and Austria. Approximately 40% of all patients advised by *Ferti*PROTEKT centers had breast cancer [1].

The 10-year survival rate of all women and stages is 86%. Women under 35 years of age have a significantly worse prognosis regarding both overall survival and the development of a relapse (Table 1).

Han et al. [5] examined the outcome as a function of the woman's age. In an analysis of 9885 women ≤50 years with breast cancer, they showed that overall survival decreased significantly at the age of <35 years. The survival rate decreased arithmetically by 5% per lower year of life for women <35 years of age (calculated until the age of approx. 25 years).

P. Sager (✉)
Breast Centre Bern Biel, Hirslanden Campus Bern, Bern, Switzerland
e-mail: patrizia.sager@hirslanden.ch

M. Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

T. Fehm
Department of Gynaecology & Obstetrics, Heinrich-Heine University Düsseldorf (HHU), Düsseldorf, Germany
e-mail: Tanja.Fehm@med.uni-duesseldorf.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_6

Table 1 Recent large studies investigating the impact of age on breast cancer prognosis by comparing two different age groups (mod. [2])

	Age, years (*n*)	Age, years (*n*)	Outcome definition	Impact of young age on outcome (multivariate model)	
				Hazard ratio	95% CI
Gnerlich et al. 2009 [3]	<40 (15548)	≥40 (227464)	Breast cancer-specific survival	1.39	1.34–1.45
Fredholm et al. 2009 [4]	<35 (378)	50–69 (13486)	Breast cancer-specific survival	1.76	1.36–2.28
Han et al. 2010 [5]	<35 (1443)	40–50 (6335)	Overall survival	30–34 y.: 1.43; 26–29 y.: 1.97	1.18–1.74; 1.48–2.62
Azim et al. 2012 [6]	≤40 (339)	>40 (2562)	Relapse free survival	1.34	1.10–1.63

Table 2 Survival rate in relation to disease stage at diagnosis in women <40 years, SEER 1988–2003 (mod. [3])

Stage	Number (*n*)	Alive (%)	Death due to breast cancer (%)	Death due to other causes (%)	TNM (UICC)
In situ	1806	98.2	0.6	1.2	Tis, N0, M0
I	4028	92.1	6.8	1.1	T1, N0, M0
II	7016	77.4	20.3	2.3	T0, T1, N1, M0 T2, N0, M0 T2, N1, M0 T3, N0, M0
III	1292	53.1	43.7	3.3	T0-T2, N2, M0 T3, N1, N2, M0 T4, N0-N2, M0 all T, N3, Mo
IV	551	27.4	66.4	6.2	All T, all N, M1
Unstaged	855	72.7	23.3	4.0	
Overall	15,548	79.6	18.3	2.2	

The outcome also depends largely on the tumour stage. In one study, the mortality of 243,012 women <40 years of age who were diagnosed breast cancer between 1998 and 2003 was analysed based on an American registry (SEER) (Table 2).

The patient's prognosis also depends on the intrinsic subtype. Young women are more likely to develop triple-negative breast cancer (=no therapy-relevant expression of the estrogen and progesterone receptor and growth factor receptor 2 = HER2), which exhibits a more aggressive course than with an expression of the factors. In addition, the prognosis of the so-called luminal B type (classification based on the gene expression profile) and the HER2 type is worse in younger patients than in older ones.

Young women with stage I hormone receptor (HR) positive high grade breast cancer have disproportionally lower 10-year survival (Fig. 1). Long-lasting estrogen deprivation therapies increase the survival rate, which however has a negative impact on bone, cardiac and reproductive health.

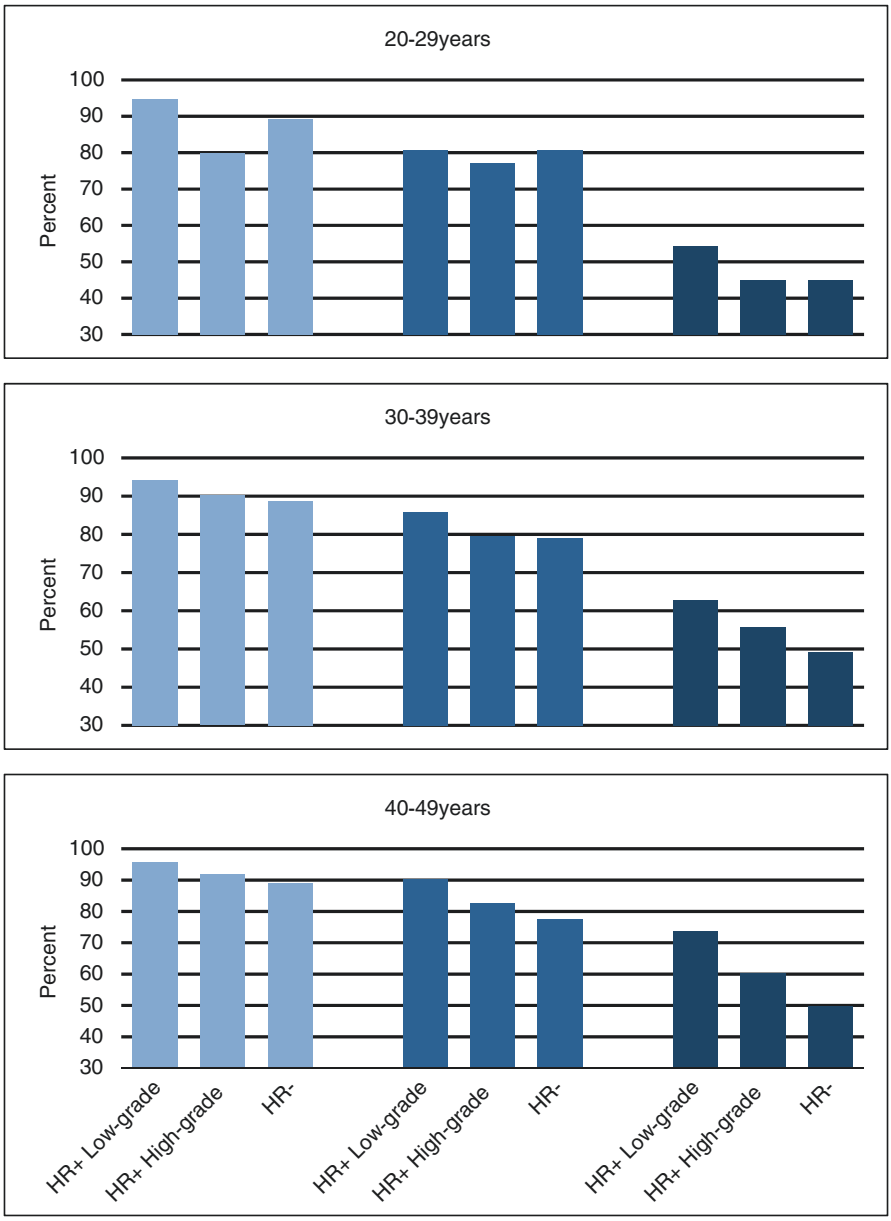


Fig. 1 Ten-year survival rate by age, stage, hormone receptor (HR) status and grade among 164.963 women diagnosed breast cancer 2000–2014 according to SEER 18 registries [7]. *Light blue*: Stage I, *Medium blue*: Stage II, *Dark Blue*: Stage III

Table 3 Risk of amenorrhoea with different chemotherapies and age groups (mod. [9])

Treatment[a]	Age < 30 years	Age 31–35 years	Age 36–40 years	Age > 40 years
CMF	4–19%	30–40%	50	80–100%
Chemotherapy with A	0%	33%		96–100%
AC-T ± H	9–20%	19–47%	21–61%	No data
M, F, Tamoxifen	Low risk			
Monoclonal antibodies	Risk unknown			

[a]C = Cyclophosphamide, M = Methotrexate, F = 5-Fluorouracil, A = Doxorubicin, T = Paclitaxel, H = Trastuzumab

Treatment Gonadotoxicity

Cytotoxically induced amenorrhea in patients after adjuvant systemic therapy is largely dependent on the age of the patient and the chemotherapy regime.

Cyclophosphamide has a particularly negative effect on the ovarian reserve, and chemotherapy-containing cyclophosphamide is associated with a risk of amenorrhoea of at least 20% in women aged around >30 years (Table 3).

When deciding to use a fertility-preservation measure, it should be noted that the risk of premature ovarian insufficiency (POI) is sometimes not very high in breast cancer; however, endocrine treatment is often given for longer, especially in estrogen-sensitive breast cancer [8]. Meanwhile, the patient's ovarian reserve can decrease further and her age can increase to such an extent that a spontaneous pregnancy is no longer possible. Therefore, even if the risk of POI is not very high, fertility-preservation measures should be considered. This is particularly true if the woman is ≥40 years old at the expected time of pregnancy, as the oocyte quality (increasing aneuploidy rate with age) also decreases significantly at this age.

Probability of Ovarian Metastasis

The data on the probability of ovarian metastasis in sporadically occurring breast cancer are not very extensive. Ovarian metastases were found in 5 of 20 women (25%) who underwent bilateral ovariectomy in 1987–1993 as treatment for metastatic breast cancer [10]. The risk is particularly high for infiltrating lobular carcinoma, where ovarian metastases were found in 5 of 14 women who died of breast cancer and were autopsied, compared to 2 of 75 (2.6%) women with infiltrating ductal carcinoma [11]. This data show that there is a relevant risk of ovarian metastases in women with higher stages of breast cancer. Stage IV breast cancers, i.e. those with metastases, have therefore been classified as diseases with an increased

Table 4 Risk of transferring malignant breast cancer cells with transplanted frozen ovarian tissue

Study	Number of patients (*n*)	Stage	Metastases found
Sanchez-Serrano et al. 2009 [12]	63	I: *n* = 16, II: *n* = 41, IIIA: *n* = 6	No
Rosendahl et al. 2011 [13]	51	II/III: *n* = 44	No
Hoekmann et al. 2015 [14]	23	I: *n* = 6, II A/B: *n* = 15, IIIA: *n* = 2	No

risk of ovarian metastasis (see chapter "Removal of Ovarian Tissue", Table 2) and cryopreservation of ovarian tissue should not be performed.

Several studies have attempted to detect breast cancer metastases in cryopreserved ovarian tissue using microscopy and immunohistochemistry. Most of these studies analysed only a small piece of the preserved tissue (Table 4).

Breast cancer metastases could not be identified in any of the studies.

In summary, the risk of ovarian metastases is low at stage N0. If the lymph nodes are positive and there is no proven peripheral metastasis (stages II and III), ovarian metastasis is also unlikely. However, the data situation here is limited. In principle, histological examination of a tissue sample should be carried out before transplantation of the tissue. With peripheral metastasis, i.e. in stage IV, cryopreservation of ovarian tissue should not be carried out, especially since the prognosis of the patient is then often too poor to justify the risk of a laparoscopy.

Regarding the risk that ovarian carcinoma could later develop from the transplanted ovarian tissue, the following should be noted: Women with a BRCA1 or BRCA2 gene mutation have a lifetime risk of developing ovarian cancer of 15–56% [15]. When performing a prophylactic adnexectomy, occult ovarian cancer was found in 6% of BRCA1 and 2% of BRCA2 carriers [16].

These data mean that cryopreservation and transplantation of ovarian tissue can be carried out after an appropriate risk assessment of a BRCA mutation, but that the tissue should be removed after pregnancy and delivery.

Effectiveness and Risks of Fertility Protection

Effectiveness

The ovarian reserve is not usually affected in women with breast cancer. The effectiveness of fertility preservation measures and the number of oocytes during ovarian stimulation are therefore not reduced [17] and only depend on age or the individual ovarian reserve. The effectiveness therefore corresponds to the information in

chapters “Ovarian Stimulation to Collect Oocytes” and “Cryopreservation of Unfertilized and Fertilized Oocytes”.

However, an exception seems to be women with a BRCA mutation. The vast majority of studies conducted so far have shown a lower number of oocytes in ovarian stimulation of mutation carriers. According to Derks-Smeets et al. [18] and Turan et al. [19], the average number of oocytes in mutation carriers is 9.0 and 7.4 and in non-mutation carriers 10.0 and 10.6. Derks-Smeets et al. [18] described this effect as particularly pronounced in BRCA1 mutation carriers. The lower number of retrieved oocytes is probably due to a lower ovarian reserve. Son et al. [20] found a 32% lower AMH concentration in mutation carriers. It is not yet known whether this also leads to lower effectiveness of cryopreservation and transplantation of ovarian tissue.

Risks from Postponement of Chemotherapy

This risk does not usually exist for breast cancer, since the time available between diagnosis and the start of chemotherapy is usually enough to carry out a fertility-preservation measure. Therefore, from a time perspective, all the measures mentioned in chapters “Ovarian Stimulation to Collect Oocytes”, “Cryopreservation of Unfertilized and Fertilized Oocytes”, “Removal of Ovarian Tissue” and “Transportation, Cryopreservation and Storage of Ovarian Tissue” can usually be carried out. Double stimulation (see Chapter “Ovarian Stimulation to Collect Oocytes”) is also possible to double the success rate, especially in low responders.

Risks from Ovarian Stimulation

In theory, there is a risk that hormone receptor-positive tumour cells could proliferate as a result of increased estrogen blood concentration due to gonadotropin stimulation to obtain oocytes (see chapter “Ovarian Stimulation to Collect Oocytes”). So far, there is no evidence for or against such an effect. However, it is very unlikely that gonadotropin stimulation influences the patient’s prognosis for the following reasons:

1. Aromatase inhibitors can also be administered alongside gonadotropin stimulation (see Chapter “Ovarian Stimulation to Collect Oocytes”), which roughly halves the serum estrogen concentration.
2. Estrogen concentrations only rise to supraphysiological concentrations over 1 week.

3. After the diagnosis has been made, the oncologist may allow several weeks to elapse before chemotherapy is started if the menstrual cycle is still normal with estrogen production. This waiting period with physiological estrogen production does not seem to have an adverse effect on the patient's prognosis.

Although the risk of the prognosis being worsened by stimulation is likely to be low, alternative fertility-preservation measures such as cryopreservation of ovarian tissue should always be considered for patients with hormone receptor-positive cancer.

Risks and Benefits of GnRH Agonists (GnRHa)

Use of GnRHa has long been considered critical. Data from the SOFT and TEXT studies [21, 22] showed that giving GnRHa for chemotherapy or endocrine therapy made no difference to the patients' prognosis. Extended GnRHa administration for adjuvant anti-hormonal therapy in premenopausal patients is now recommended in the current guidelines.

Several randomised studies have now also demonstrated the fertility preservation benefits of GnRHa [23–27]. Reduction in amenorrhoea and increase in pregnancy rates were confirmed in meta-analyses (see chapter "GnRH Agonists").

As a result, the administration of GnRHa during chemotherapy has now been accepted by German oncologists (Study Group for Gynaecological Oncology, AGO; The German Society for Gynaecology and Obstetrics, DGGG; Guidelines of the European Society for Medical Oncology, ESMO) as an option for fertility preservation.

GnRHa can therefore also be offered as a fertility-preservation measure to patients with hormone receptor-positive breast cancer.

GnRHa could also be beneficial in premenopausal patients – regardless of a prospective desire to have children. The lower probability of POI reduces the risk of menopausal symptoms. It should be noted that hormone replacement therapy is contraindicated in women with breast cancer (see chapter "Premature Ovarian Insufficiency: Hormone Replacement Therapy and Follow Up").

Practical Approach

Patients should attend a reproductive medicine centre as early as possible so that enough time is available for the implementation of fertility-preservation measures. They can also attend before staging is completed (Fig. 2).

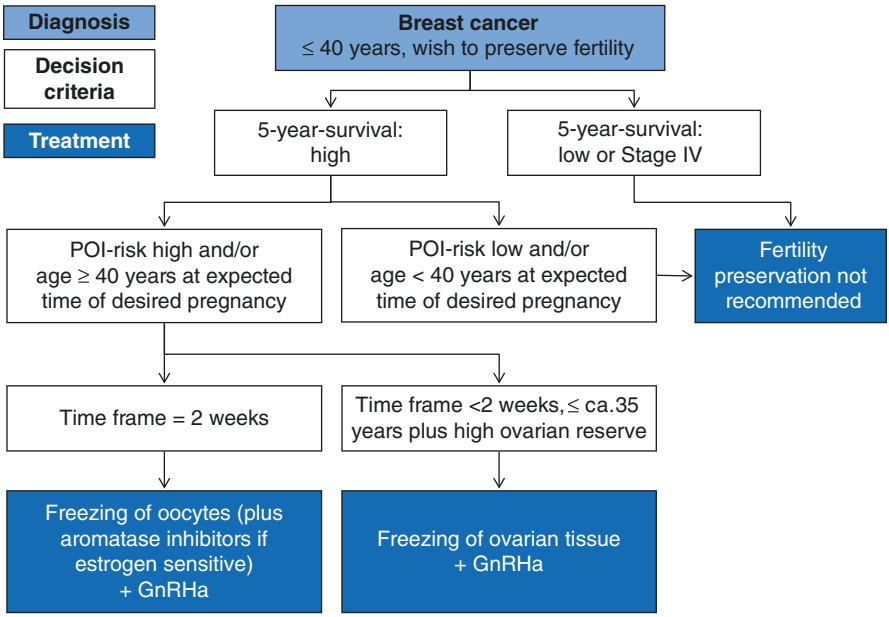


Fig. 2 Algorithm for fertility preservation in breast cancer patients

References

1. von Wolff M, Dittrich R, Liebenthron J, Nawroth F, Schüring AN, Bruckner T, Germeyer A. Fertility-preservation counselling and treatment for medical reasons: data from a multinational network of over 5000 women. Reprod Biomed Online. 2015;31:605–12.
2. Azim HA Jr, Partridge AH. Biology of breast cancer in young women. Breast Cancer Res. 2014;4:427.
3. Gnerlich JL, Deshpande AD, Jeffe DB, Sweet A, White N, Margenthaler JA. Elevated breast cancer mortality in women younger than age 40 years compared with older women is attributed to poorer survival in early-stage disease. J Am Coll Surg. 2009;208:341–7.
4. Fredholm H, Eaker S, Frisell J, Holmberg L, Fredriksson I, Lindman H. Breast cancer in young women: poor survival despite intensive treatment. PLoS One. 2009;4:e7695.
5. Han W, Kang SY. Korean Breast Cancer Society. Relationship between age at diagnosis and outcome of premenopausal breast cancer: age less than 35 years is a reasonable cut-off for defining young age-onset breast cancer. Clin Breast Cancer Res Treat. 2010;119:193–200.
6. Azim HA Jr, Michiels S, Bedard PL, Singhal SK, Criscitiello C, Ignatiadis M, Haibe-Kains B, Piccart MJ, Sotiriou C, Loi S. Elucidating prognosis and biology of breast cancer arising in young women using gene expression profiling. Clin Cancer Res. 2012;18:1341–51.
7. Thomas A, Anthony R, Pinkerton E, Schroeder MC, Conway KM, Hundley G, LR MN, Oleson J, Lynch CH, Romitti P. Incidence and survival among young women with Stage I-III breast cancer: SEER 2000–2015. JNCI Cancer Spectr. 2019;7:pkz040.
8. Goldhirsch A, Gelber RD, Castiglione M. The magnitude of endocrine effects of adjuvant chemotherapy for premenopausal breast cancer patients: the International Breast Cancer Study Group. Ann Oncol. 1990;1:183–8.

9. Waks AG, Partridge AH. Fertility preservation in patients with breast cancer: necessity, methods, and safety. J Natl Compr Cancer Netw. 2016;14:355–63.
10. Curtin JP, Barakat RR, Hoskins WJ. Ovarian disease in women with breast cancer. Obstet Gynecol. 1994;84:449–52.
11. Harris M, Howell A, Chrissohou M, Swindell RI, Hudson M, Sellwood RA. A comparison of the metastatic pattern of infiltrating lobular carcinoma and infiltrating duct carcinoma of the breast. Br J Cancer. 1984;50:23–30.
12. Sánchez-Serrano M, Novella-Maestre E, Roselló-Sastre E, Camarasa N, Teruel J, Pellicer A. Malignant cells are not found in ovarian cortex from breast cancer patients undergoing ovarian cortex cryopreservation. Hum Reprod. 2009;24:2238–43.
13. Rosendahl M, Timmermans Wielenga V, Nedergaard L, Kristensen SG, Ernst E, Rasmussen PE, Anderson M, Schmidt KT, Andersen CY. Cryopreservation of ovarian tissue for fertility preservation: no evidence of malignant cell contamination in ovarian tissue from patients with breast cancer. Fertil Steril. 2011;95:2158–61.
14. Hoekman EJ, Smit VT, Fleming TP, Louwe LA, Fleuren GJ, Hilders CG. Searching for metastases in ovarian tissue before autotransplantation: a tailor-made approach. Fertil Steril. 2015;103:469–77.
15. Bougie O, Weberpals JI. Clinical considerations of BRCA1- and BRCA2-mutation carriers: a review. Int J Surg Oncol. 2011;2011:374012.
16. Finch A, Shaw P, Rosen B, Murphy J, Narod SA, Colgan TJ. Clinical and pathologic findings of prophylactic salpingo-oophorectomies in 159 BRCA1 and BRCA2 carriers. Gynecol Oncol. 2006;100:58–64.
17. von Wolff M, Bruckner T, Strowitzki T, Germeyer A. Fertility preservation: ovarian response to freeze oocytes is not affected by different malignant diseases - an analysis of 992 stimulations. J Assist Reprod Genet. 2018;35:1713–9.
18. Derks-Smeets IAP, van Tilborg TC, van Montfoort A, Smits L, Torrance HL, Meijer-Hoogeveen M, Broekmans F, Dreesen JCFM, Paulussen ADC, Tjan-Heijnen VCG, Homminga I, van den Berg MMJ, Ausems MGEM, de Rycke M, de Die-Smulders CEM, Verpoest W, van Golde R. BRCA1 mutation carriers have a lower number of mature oocytes after ovarian stimulation for IVF/PGD. J Assist Reprod Genet. 2017;34:1475–82.
19. Turan V, Bedoschi G, Emirdar V, Moy F, Oktay K. Ovarian stimulation in patients with cancer: impact of letrozole and BRCA mutations on fertility preservation cycle outcomes. Reprod Sci. 2018;25:26–32.
20. Son KA, Lee DY, Choi D. BRCA1 mutation carriers have a lower number of mature oocytes after ovarian stimulation for IVF/PGD. Association of BRCA mutations and anti-müllerian hormone level in young breast cancer patients. Front Endocrinol (Lausanne). 2019;10:235.
21. Regan MM, Walley BA, Francis PA, Fleming GF, Láng I, Gómez HL, Colleoni M, Tondini C, Pinotti G, Salim M, Spazzapan S, Parmar V, Ruhstaller T, Abdi EA, Gelber RD, Coates AS, Goldhirsch A, Pagani O. Concurrent and sequential initiation of ovarian function suppression with chemotherapy in premenopausal women with endocrine-responsive early breast cancer: an exploratory analysis of TEXT and SOFT. Ann Oncol. 2017;28:2225–32.
22. Bernhard J, Luo W, Ribi K, Colleoni M, Burstein HJ, Tondini C, Pinotti G, Spazzapan S, Ruhstaller T, Puglisi F, Pavesi L, Parmar V, Regan MM, Pagani O, Fleming GF, Francis PA, Price KN, Coates AS, Gelber RD, Goldhirsch A, Walley BA. Patient-reported outcomes with adjuvant exemestane versus tamoxifen in premenopausal women with early breast cancer undergoing ovarian suppression (TEXT and SOFT): a combined analysis of two phase 3 randomised trials. Lancet Oncol. 2015;16:848–58.
23. Moore HC, Unger JM, Phillips KA, Boyle F, Hitre E, Porter D, Francis PA, Goldstein LJ, Gomez HL, Vallejos CS, Partridge AH, Dakhil SR, Garcia AA, Gralow J, Lombard JM, Forbes JF, Martino S, Barlow WE, Fabian CJ, Minasian L, Meyskens FL Jr, Gelber RD, Hortobagyi GN, Albain KS. POEMS/S0230 investigators. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy. N Engl J Med. 2015;372:923–32.

24. Moore HCF, Unger JM, Phillips KA, Boyle F, Hitre E, Moseley A, Porter DJ, Francis PA, Goldstein LJ, Gomez HL, Vallejos CS, Partridge AH, Dakhil SR, Garcia AA, Gralow JR, Lombard JM, Forbes JF, Martino S, Barlow WE, Fabian CJ, Minasian LM, Meyskens FL, Gelber RD, Hortobagyi GN, Albain KS. Final analysis of the Prevention of Early Menopause Study (POEMS)/SWOG Intergroup S0230. J Natl Cancer Inst. 2019;111:210–3.
25. Paluch-Shimon S, Pagani O, Partridge AH, Abulkhair O, Cardoso MJ, Dent RA, Gelmon K, Gentilini O, Harbeck N, Margulies A, Meirow D, Pruneri G, Senkus E, Spanic T, Sutliff M, Travado L, Peccatori F, Cardoso F. ESO-ESMO 3rd international consensus guidelines for breast cancer in young women (BCY3). Breast. 2017;35:203–17.
26. Burstein HJ, Temin S, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Rowden D, Solky AJ, Stearns V, Winer EP, Griggs JJ. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: American Society of Clinical Oncology clinical practice guideline focused update. J Clin Oncol. 2014;32:2255–69.
27. Gori S, Puglisi F, Cinquini M, Pappagallo G, Frassoldati A, Biganzoli L, Cortesi L, Fiorentino A, Angiolini C, Tinterri C, De Censi A, Levaggi A, Del Mastro L. Adjuvant endocrine therapy in premeno-pausal patients with hormone receptor-positive early breast cancer: evidence evaluation and GRADE recommendations by the Italian Association of Medical Oncology (AIOM). Eur J Cancer. 2018;99:9–19.

Hodgkin's Lymphoma

Carolin Bürkle, Michael von Wolff, and Karolin Behringer

Stage-Dependent Prognosis

Hodgkin's lymphoma occurs worldwide with an annual incidence of 2–3/100,000, with men being affected slightly more frequently in a ratio of 3:2 [1].

A total of 2490 new cases were recorded in Germany in 2016. Young people are frequently affected and up to ¾ of patients are under 60 years of age at diagnosis [2]. For young patients, family planning is often not yet complete at the time of the initial diagnosis or has not been addressed at all. About 30% of women counselled in the *Ferti*PROTEKT network suffer from lymphoma, predominantly Hodgkin's lymphoma [3].

In recent decades, Hodgkin's lymphoma has developed from an incurable disease to one of the best treatable oncological diseases in adulthood with outstanding 5-year survival rates (Tables 1 and 2).

In a large retrospective study by Glimelius et al [4], the outcome of a total of 1947 Swedish Hodgkin's lymphoma patients diagnosed in the period 1992–2009 and aged between 15 and 59 years at diagnosis was recorded. The age distribution in the patient-collective showed the expected age distribution with 36.4% in the youngest

C. Bürkle (✉)
Internal Medicine Department III, Hematology and Oncology, University Hospital of Munich, Großhadern, Munich, Germany
e-mail: Carolin.Buerkle@med.uni-muenchen.de

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

K. Behringer
Internal Medicine Department I, Hematology and Oncology, University Hospital of Cologne, Cologne, Germany
e-mail: karolin.behringer@uk-koeln.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_7

Table 1 Survival rates of women and men depending on age

Age at initial diagnosis (years)		5 years relative survival rate (95% confidence interval)	15 years relative survival rate (95% confidence interval)
Glimelius et al. [4]	18–29	96% (95–98%)	94% (91–95%)
	30–39	95% (93–97%)	91% (87–94%)
	40–49	93% (90–96%)	87% (81–91%)
Pulte et al. [5]	15–29	97.9%	
	30–39	95.8%	
	40–59	88.3%	

Table 2 Survival rates of women and men depending on initial disease stage

Stage		Therapy	5 years FFTF (95% confidence interval)	5 years PFS (95% confidence interval)	5 years OS (95% confidence interval)
Behringer et al. 2015 [7]	Early favourable	2 cycles of ABVD	93.1% (90.7–95.5%)	93.5% (91.1–95.9%)	97.6% (96.1–99.1%)
von Tresckow et al. 2012 [8]	Early un-favourable	2 cycles of escalated BEACOPP +2 cycles of ABVD	94.8% (93.1–96.6%)	95.4% (93.7–97.1%)	97.2% (95.8–98.6%)
Engert et al. 2012 [9]	Advanced	6 cycles of escalated BEACOPP	89.3% (86.5–92.1%)	90.3% (87.6–93.0%)	95.3% (93.4–97.2%)

Outcome criteria: *FFTF* freedom from treatment failure, *PFS* progression-free survival and *OS* overall survival

age group of 18–29 years, 21.2% aged 30–39 years and 14.2% aged 40–49 years. The outcome in terms of age is shown with the 5- and 15-year (relative) survival rates and shows a clear age-dependent outcome. The 15-year survival rate is 94% in the youngest age group of 18–29 years, and 87% in the 40–49 years age group.

This is also the result of an evaluation by Pulte et al. [5], in which the 5-year (relative) survival of a total of 5300 patients suffering from Hodgkin's lymphoma in Germany from 1997 to 2006 is evaluated. The 5-year survival rate also decreased with increasing age.

As a limitation, it should be mentioned that there were major therapeutic advances in the studied period of 18 or 10 years, and that the patient collective was treated with various radiation and chemotherapy regimens. The cure rate also depends on the stage, the response to therapy and the risk factors (Table 2). Overall, it is between 80 and 95%, and the number of long-term survivors is steadily increasing [6].

After staging, patients are divided into three risk groups using the Ann Arbor classification (describes the involvement of the lymph node regions) and the presence or absence of certain risk factors:

1. Clinical stages (CS) I-IIA and B without risk factors are considered early, prognostically favourable stages.
2. CS IA and B and CS IIA with ≥1 risk factor or CS IIB with the risk factors: accelerated blood sedimentation rate and/or ≥3 affected lymph node areas are listed as an intermediate patient group.
3. CS II B with the risk factors: large mediastinal tumour mass and/or extranodal tumour foci as well as CS III/IV are classified as advanced stages.

According to the German Swiss Austrian S3 guideline "Diagnosis, treatment and follow-up care of Hodgkin's lymphoma in adult patients" from June 2018, the stage-adapted treatment recommendation for patients ≤60 years of age is as follows [10]:

- In the early stages, two cycles of ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) and then involved field (IF)—RT with 20 Gray are administered.
- In the intermediate stages, the recommended treatment regimen consists of combination chemotherapy consisting of two cycles of escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone) and two ABVD (2 + 2) cycles, as well as subsequent IF-RT with 30 Gray.
- In advanced stages, intermediate staging with PET/CT is carried out after two BEACOPP cycles. PET-negative patients receive two additional escalated BEACOPP cycles, for a total of four cycles. PET/CT positive patients receive four further escalated BEACOPP cycles, for a total of six cycles. If PET-positive residual lymphoma tissue of ≥2.5 cm is still present after the end of chemotherapy, localized radiotherapy with 30 Gray is carried out.

In the analyses of the HD13 trial by the German Hodgkin's Study Group (GHSG) for the early stages, HD14 for the intermediate stages and HD15 and HD18 for the advanced stages, the current standard treatments showed excellent outcome results in all stages. The values collected refer to a follow-up period of 5 years, in which the following outcome criteria are recorded: "freedom from treatment failure" (FFTF), "progression-free survival" (PFS), which records patients who survived 5 years progression-free/relapse-free and 5-year overall survival (OS), which represents overall survival after 5 years. There was a stage-dependent outcome with a 5-year PFS and OS of 93.5% and 97.6% in the early stages versus 90.3% and 95.3% in the advanced stages.

Treatment Gonadotoxicity

Chemotherapy and radiotherapy always carry a risk of gonadotoxicity. The chemotherapy regimens administered for Hodgkin's lymphoma show dose- and substance-dependent gonadotoxicity, with the more intensive escalated BEACOPP regime having a higher gonadotoxic effect than the ABVD regimen. Of the active

ingredients administered, the alkylating agents procarbazine and cyclophosphamide play a decisive role [11–13].

Data on fertility after Hodgkin's lymphoma treatment were obtained in two studies by the German Hodgkin's Study Group (GHSG). The results published by Behringer et al. [14] looked at 405 patients who were under 40 years of age at first diagnosis and were treated in the third study generation (HD7–nine studies) between 1994 and 1998. The more recent data from Behringer et al. [15] were obtained from a follow-up of a total of 1323 male and female patients from the fifth study generation (HD13–15 studies).

Treatment Gonadotoxicity in Women

The publication by Behringer et al. [14], with a mean follow-up time of 3.2 years, showed that 51.4% of patients who received eight cycles of escalated BEACOPP suffered from amenorrhea. The most frequent reports of amenorrhea were from women in advanced stages, when the age at initial diagnosis was ≥30 years and when no oral contraceptives were taken during chemotherapy.

With a mean follow-up time of 46 months in a total of 562 female Hodgkin's lymphoma survivors who were <40 years old at initial diagnosis, the evaluation by Behringer et al. [15] also showed a clear difference with regard to age at initial diagnosis (</≥30 years) and the treatment regimen administered (ABVD or escalated BEACOPP) (Tables 3 and 4).

The measured values for follicle-stimulating hormone (FSH) and anti-Müllerian hormone (AMH) were in women ≥30 years and were significantly worse after treatment with escalated BEACOPP, consistent with damage to the ovarian reserve. The occurrence of regular menstruation was reported by more than 90% of women after early-stage therapy. In most cases, it started within the first year after treatment. A recent paper by Weibull et al. showed similar conclusions [16]. Fortunately, a comparable birth rate between the examined 449 relapse-free Hodgkin lymphoma

Table 3 Hormone and menstrual cycle parameters in women age 18–29 years after different chemotherapy regimens

		2 cycles of ABVD	2 cycles of ABVD plus 2 cycles of escalated BEACOPP	6 cycles of escalated BEACOPP
AMH (µg/L) (95% confidence interval)		2.2 (1.4–3.6)	0.9 (0.7–1.2)	0.1 (0.1–0.2)
FSH (U/L) (95% confidence interval)		2.4 (1.2–4.7)	4.4 (3.2–6.1)	10.6 (6.3–18.0)
Regular cycle	After therapy	94%	100%	88%
	At the time of analysis (Ø 46 months after therapy)	88%	95%	81%

Table 4 Hormone and menstrual cycle parameters in women age 18–29 years after different chemotherapy regimens

		2 cycles of ABVD	2 cycles of ABVD plus 2 cycles of escalated BEACOPP	6 cycles of escalated BEACOPP
AMH (µg/L) (95% confidence interval)		0.7 (0.3–1.6)	0.0 (0.0–0.1)	0.0 (0.0–0.0)
FSH (U/L) (95% confidence interval)		7.5 (5.9–9.6)	11.8 (8.2–16.9)	23.6 (14.6–38.2)
Regular cycle	After therapy	97%	90%	55%
	At the time of analysis (Ø 46 months after therapy)	95%	75%	40%

Table 5 Rate of amenorrhea depending on chemotherapy and age of female survivors [15]

Chemotherapy	Age 18–29 years (%)	Age 30–45 years (%)
2 cycles of A(B)VD	11	8
4 cycles of ABVD	10	16
2 cycles of ABVD plus 2 cycles of escalated BEACOPP	7	25
6 cycles of escalated BEACOPP	24	74
8 cycles of escalated BEACOPP	20	70

patients (all stages) and the normal population was shown 3 years after diagnosis. The birth rate was 22.5% in the group of former patients.

Data from Behringer et al. [15] showed a longer time for ovarian function recovery after treatment with six to eight cycles of escalated BEACOPP, which was strongly dependent on the age of the patient at initial diagnosis. The risk of persistent amenorrhoea after 4 years was 25% in 25-year-old patients, while it increased to 50% in 30-year-old patients.

Table 5 summarises the risks of amenorrhoea after a mean follow-up period of 46 months after chemotherapy [15] and thus the risk of POI depending on the chemotherapy used and the age of the woman and, derived from this, the recommendations for the implementation of a fertility preservation measure are shown in Fig. 1.

Treatment Gonadotoxicity in Men

Radiochemotherapy mainly affects spermatogenesis in men, which is often limited even before the start of therapy, especially in the advanced stages [17]. The effects of treatment on testosterone production, however, are small. In the study data collected by Behringer et al. [15], the mean values for testosterone were within the limits after all treatment intensities.

Table 6 Hormone parameters of male survivors (age 18–49 years) depending on the type of chemotherapy regimen

Hormone parameters	2 cycles of ABVD	2 cycles of ABVD plus 2 cycles of escalated BEACOPP	6 cycles of escalated BEACOPP
Mean FSH (U/L) (95% confidence interval)	5.6 (4.8–6.6)	7.9 (6.8–9.1)	18.7 (17.3–20.3)
FSH > 12.4 U/L	13%	29%	80%
Mean inhibin B (ng/L) (95% confidence interval)	126.1 (111.5–140.7)	93.3 (80.5–107.2)	16.9 (12.9–20.9)
Inhibin B < 25 ng/L	7%	22%	75%
Mean LH (U/L) (95% confidence interval)	4.8 (4.3–5.3)	5.1 (4.6–5.6)	7.0 (6.6–7.5)
LH > 8.6 U/L	11%	13%	28%
Mean testosterone (ng/L) (95% confidence interval)	4.2 (3.9–4.6)	4.7 (4.4–5.0)	4.1 (3.8–4.4)
Testosterone <2.8 ng/L	20%	14%	21%

Table 7 Risk of azoospermia depending on the chemotherapy regimen [18]

Chemotherapy	Number of male survivors with azoospermia/total score (*n*)	Percentage of male survivors with azoospermia (%)
2–8 cycles of ABVD	0/202	0
2–6 cycles of ABVD/COPP or OPP or MOPP	11/13	84.6
2–6 cycles of ABVD plus inguinal radiotherapy (30–40 Gy)	3/13	23
2–8 cycles of BEACOPP	8/16	50

Behringer et al. [15] examined hormone parameters depending on the chemotherapy regimen (Table 6). Seven hundred and sixty-one male Hodgkin's lymphoma survivors who were younger than 50 years at the time of initial diagnosis were examined after a mean follow-up period of 48 months. Inhibin B and FSH levels correlated significantly with the intensity of chemotherapy (Table 6). After treatment in the early stages, 50% of the men showed an Inhibin B/FSH ratio corresponding with definite fertility (inhibin B/FSH ratio > 23.5 ng/U). However, the highest proportion of inhibin B/FSH values, which correlated with oligospermia, was found after six to eight cycles of escalated BEACOPP therapy (88.8%).

Paoli et al. [18] examined sperm parameters and azoospermia rates depending on different chemotherapy regimens (Table 7). Chemotherapy according to the ABVD regimen led to a significant reduction in sperm concentration, which normalised within 24 months. Other chemotherapy regimens and radiotherapy often lead to long-term azoospermia (Table 6). Although the cohorts in the study by Paoli et al. are small, they show a clear correlation between the intensity of the chemotherapy regimen and sometimes long-term azoospermia rates.

Table 8 Trials examining ovarian contamination of cryopreserved ovarian tissue samples with Hodgkin's lymphoma cells

Trial	Number of female survivors (*n*)	Stage of illness	Metastases proven
Seshadri et al. 2006 [19]	26 (24 with information about stage of illness)	I/II: *n* = 15 III/IV: *n* = 9	No
Meirow et al. 2008 [20]	33	IV: *n* = 8	No
Bittinger et al. 2011 [21]	1	IIIB	Yes
Hoekmann et al. 2015 [22]	6	IIA: *n* = 3 IIIB: *n* = 1 IVB: *n* = 1	No

Table 7 summarises the risks of long-term azoospermia 2 or 3 years after treatment [18], depending on the chemotherapy used. Recommendations for the implementation of a fertility-preservation measure are shown in Fig. 2.

Probability of Gonadal Metastasis

The data available for estimating gonadal metastasis are limited.

Several studies have systematically attempted to detect tumour cells in cryopreserved ovarian tissue. Table 8 shows the studies known to the authors and published to date. Overall, there is almost never any evidence of Hodgkin's lymphoma cells in ovarian tissue, even in higher grade tumour stages. However, a case report with ovarian involvement of the lymphoma has been published [21]. Because of this case report and since it is never possible to examine the tissue to be transplanted, only an ovarian biopsy, a tissue sample should be examined histologically during cryopreservation or at the latest before transplantation.

Based on these examinations and the large number of ovarian tissue transplants without evidence of recurrence, Hodgkin's lymphoma was classified as a disease with a low risk of metastasis (see chapter "Removal of Ovarian Tissue", Table 2).

Effectiveness and Risks of Fertility Preservation

Effectiveness

Women and men with Hodgkin's lymphoma are usually of a younger age. Women therefore usually have a good ovarian reserve and oocyte quality, and fertility-protective measures such as cryopreservation of ovarian tissue and oocytes are also very effective.

However, there are studies which suggest a lower ovarian reserve in women with Hodgkin's lymphoma. The number of oocytes obtained during ovarian stimulation is 1.2–1.4 times lower in women with Hodgkin's lymphoma [3, 23]. Lawrenz et al. [24] demonstrated a 35% lower serum concentration of AMH in women with Hodgkin's lymphoma, which explains the lower response to ovarian stimulation.

However, it is questionable whether the lower AMH concentration also leads the fertility-preservation measures being less effective. If oocytes are to be cryopreserved, the stimulation dose can be adjusted because the women are usually younger. If ovarian tissue is cryopreserved, the AMH concentration plays a minor role. What is important, however, is the actual ovarian reserve, i.e. the density of primordial follicles, which—in contrast to the AMH concentration—is not reduced in women with Hodgkin's lymphoma [25].

It should be noted that sperm quality is reduced in men with lymphomas. Caponecchia et al. [17] found an average sperm concentration of 34.5 million/mL in men with lymphoma (Hodgkin's and non-Hodgkin's) compared to fertile men with 46.5 million/mL. However, motility and morphology were not affected.

Risks

Fertility-preservation measures are usually only associated with minor risks in patients with Hodgkin's lymphoma. The tumour cells are not hormone-dependent and the time available for carrying out all procedures is usually sufficient. The risk of lymphoma cells appearing in the gonadal tissue also appears to be low, as no tumour cells have been detected to date.

It should be noted, however, that Hodgkin's lymphomas are often accompanied by mediastinal involvement, which means that intubation and extubation can be risky if the patient undergoes laparoscopy. In these cases, ovarian tissue should only be removed if the anaesthetists assess the sedation and intubation risk as being low. Alternatively, hormonal stimulation for cryopreservation of unfertilised or fertilised oocytes may be considered, as intubation anaesthesia is not required for follicle puncture.

Practical Approach

Patients should always be introduced to a reproductive medicine centre as early as possible, so that enough time is available for the implementation of fertility-preservation measures. This can also take place before the chemotherapy regimen has been decided upon (Figs. 1 and 2).

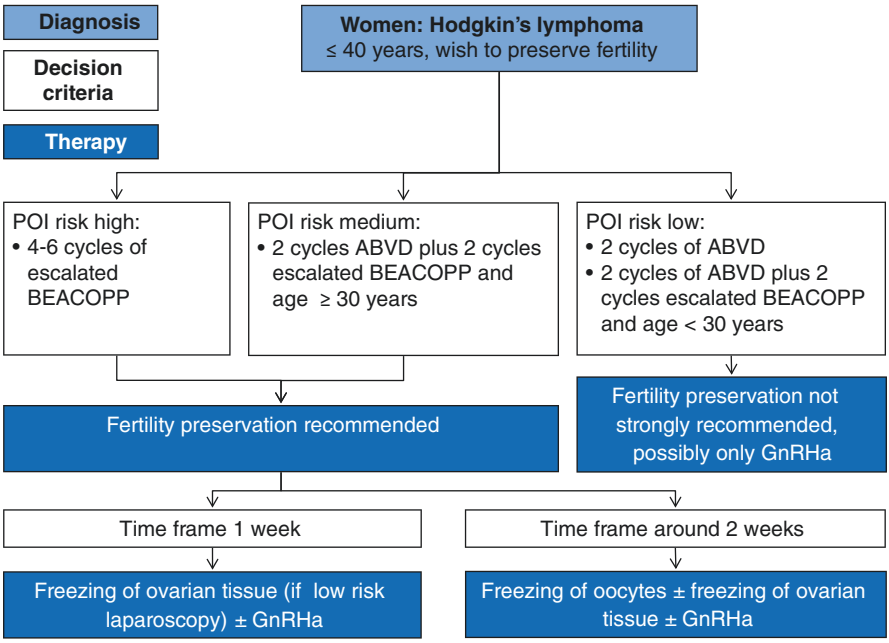


Fig. 1 Flowchart for fertility preservation in female Hodgkin's lymphoma patients

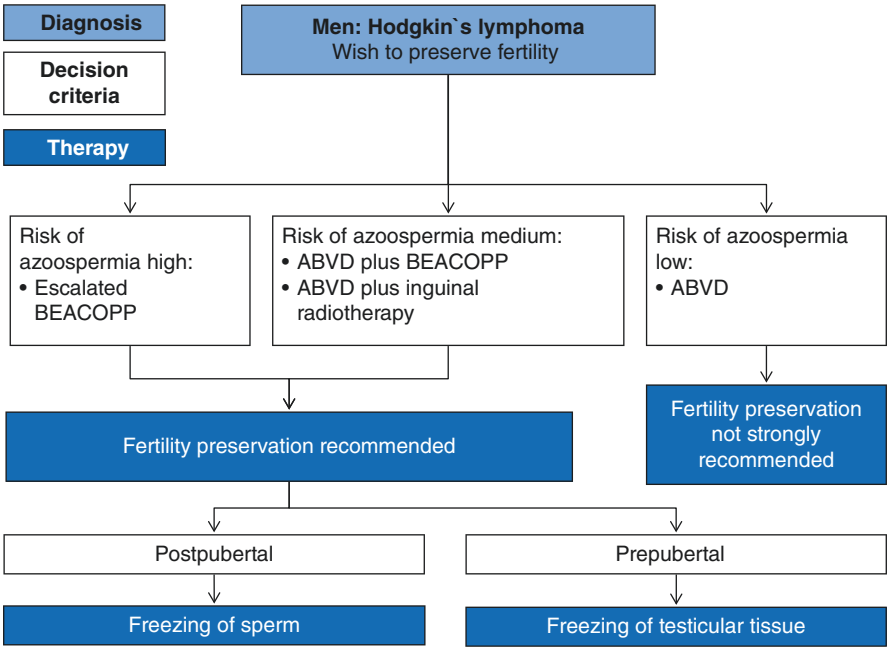


Fig. 2 Flowchart for fertility preservation in male Hodgkin's lymphoma patients

References

1. Sant M, Allemani C, Tereanu C, De Angelis R, Capocaccia R, Visser O, et al. Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. Blood. 2010;116(19):3724–34. https://doi.org/10.1182/blood-2010-05-282632.
2. Robert-Koch-Institut (2016). 3.28 Morbus Hodgkin. Krebs Deutschl. 122–5.
3. von Wolff M, Bruckner T, Strowitzki TGA. Fertility preservation: ovarian response to freeze oocytes is not affected by different malignant diseases-an analysis of 992 stimulations. J Assist Reprod Genet. 2018;35(9):1713–9.
4. Glimelius I, Ekberg S, Jerkeman M, Chang ET, Björkholm M, Andersson TML, et al. Long-term survival in young and middle-aged Hodgkin lymphoma patients in Sweden 1992-2009-trends in cure proportions by clinical characteristics. Am J Hematol. 2015;90(12):1128–34. https://doi.org/10.1002/ajh.24184.
5. Pulte D, Jansen L, Gondos A, Emrich K, Holleczek B, Katalinic A, et al. Improved population level survival in younger Hodgkin lymphoma patients in Germany in the early twenty-first century. Br J Haematol. 2014;164(6):851–7. https://doi.org/10.1111/bjh.12722.
6. Skoetz N, Trelle S, Rancea M, Haverkamp H, Diehl V, Engert A, et al. Effect of initial treatment strategy on survival of patients with advanced-stage Hodgkin's lymphoma: a systematic review and network meta-analysis. Lancet Oncol. 2013;14(10):943–52. https://doi.org/10.1016/S1470-2045(13)70341-3.
7. Behringer K, Goergen H, Hitz F, Zijlstra JM, Greil R, Markova J, et al. Omission of dacarbazine or bleomycin, or both, from the ABVD regimen in treatment of early-stage favourable Hodgkin's lymphoma (GHSG HD13): an open-label, randomised, non-inferiority trial. Lancet. 2014;385(9976):1418–27. https://doi.org/10.1016/S0140-6736(14)61469-0.
8. Von Tresckow B, Plütschow A, Fuchs M, Klimm B, Markova J, Lohri A, et al. Dose-intensification in early unfavorable Hodgkin's lymphoma: final analysis of the German Hodgkin study group HD14 trial. J Clin Oncol. 2012;30(9):907–13. https://doi.org/10.1200/JCO.2011.38.5807.
9. Engert A, Haverkamp H, Kobe C, Markova J, Renner C, Ho A, et al. Reduced-intensity chemotherapy and PET-guided radiotherapy in patients with advanced stage Hodgkin's lymphoma (HD15 trial): a randomised, open-label, phase 3 non-inferiority trial. Lancet. 2012;379(9828):1791–9. https://doi.org/10.1016/S0140-6736(11)61940-5.
10. S3-Leitlinie Diagnostik, Therapie und Nachsorge des Hodgkin Lymphoms bei erwachsenen. Patienten. 2019: 1–183.
11. van Beek RD, Smit M, van den Heuvel-Eibrink MM, de Jong FH, Hakvoort-Cammel FG, van den Bos C, et al. Inhibin B is superior to FSH as a serum marker for spermatogenesis in men treated for Hodgkin's lymphoma with chemotherapy during childhood. Hum Reprod. 2007;22(12):3215–22. https://doi.org/10.1093/humrep/dem313.
12. Kiserud CE, Fosså A, Bjøro T, Holte H, Cvancarova M, Fosså SD. Gonadal function in male patients after treatment for malignant lymphomas, with emphasis on chemotherapy. Br J Cancer. 2009;10:455–63. https://doi.org/10.1038/sj.bjc.6604892.
13. Kulkarni S, et al. Gonadal function following ABVD therapy for Hodgkin' s disease. Am J Clin Oncol. 1997;20(4):354–7.
14. Behringer K, Breuer K, Reineke T, May M, Nogova L, Klimm B. Secondary amenorrhea after Hodgkin's lymphoma is influenced by age at treatment, stage of disease, chemotherapy regimen, and the use of oral contraceptives during therapy: a report from the German Hodgkin's lymphoma study group. J Clin Oncol. 2005;23(30):7555–64. https://doi.org/10.1200/JCO.2005.08.138.
15. Behringer K, Mueller H, Goergen H, Thielen I, Eibl AD, Stumpf V, et al. Gonadal function and fertility in survivors after Hodgkin lymphoma treatment within the German Hodgkin study group HD13 to HD15 trials. J Clin Oncol. 2013;31(2):231–9. https://doi.org/10.1200/JCO.2012.44.3721.

16. Weibull CE, Eloranta S, Smedby KE, Bjorkholm M, Kristinsson SY, Johansson AL, et al. Pregnancy and the risk of relapse in patients diagnosed with Hodgkin lymphoma. J Clin Oncol. 2016;34(4):337–44. https://doi.org/10.1200/JCO.2015.63.3446.
17. Caponecchia L, Cimino G, Sacchetto R, Fiori C, Sebastianelli A, Salacone P, et al. Do malignant diseases affect semen quality? Sperm parameters of men with cancers. Andrologia. 2016;48(3):333–40.
18. Paoli D, Rizzo F, Fiore G, Pallotti F, Pulsoni A, Annechini G, et al. Spermatogenesis in Hodgkin's lymphoma patients: a retrospective study of semen quality before and after different chemotherapy regimens. Hum Reprod. 2016;31(2):263–72. https://doi.org/10.1093/humrep/dev310.
19. Seshadri T, Gook D, Lade S, Spencer A, Grigg A, Tiedemann K, et al. Lack of evidence of disease contamination in ovarian tissue harvested for cryopreservation from patients with Hodgkin lymphoma and analysis of factors predictive of oocyte yield. Br J Cancer. 2006;94(7):1007–10. https://doi.org/10.1038/sj.bjc.6603050.
20. Meirow D, Hardan I, Dor J, Fridman E, Elizur S, Ra'anani H, et al. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. Hum Reprod. 2008;23(5):1007–13. https://doi.org/10.1093/humrep/den055.
21. Bittinger SE, Nazaretian SP, Gook DA, Parmar C, Harrup RA, Stern CJ. Detection of Hodgkin lymphoma within ovarian tissue. Fertil Steril. 2011;95(2):803e3–6. https://doi.org/10.1016/j.fertnstert.2010.07.1068.
22. Hoekman EJ, Smit VTHBM, Fleming TP, Louwe LA, Fleuren GJ, CGJM H. Searching for metastases in ovarian tissue before autotransplantation: a tailor-made approach. Fertil Steril. 2015;103(2):469–77. https://doi.org/10.1016/j.fertnstert.2014.11.001.
23. Lekovich J, Lobel ALS, Stewart JD, Pereira N, Kligman I, Rosenwaks Z. Female patients with lymphoma demonstrate diminished ovarian reserve even before initiation of chemotherapy when compared with healthy controls and patients with other malignancies. J Assist Reprod Genet. 2016;33(5):657–62. https://doi.org/10.1007/s10815-016-0689-1.
24. Lawrenz B, Fehm T, Von Wolff M, Soekler M, Huebner S, Henes J, et al. Reduced pretreatment ovarian reserve in premenopausal female patients with Hodgkin lymphoma or non-Hodgkin-lymphoma—evaluation by using antimüllerian hormone and retrieved oocytes. Fertil Steril. 2012;98(1):141–4. https://doi.org/10.1016/j.fertnstert.2012.04.021.
25. Liebenthron J, Reinsberg J, van der Ven K, Saenger N, Kruessel JS, von Wolff M. Serum anti-Mullerian hormone concentration and follicle density throughout reproductive life and in different diseases—implications in fertility preservation. Hum Reprod. 2019;34(12):2513–22. https://doi.org/10.1093/humrep/dez215.

Acute Leukaemia

Michael von Wolff, Nicola Gökbuget, and Andrea Jarisch

Epidemiology and Prognosis

Acute Lymphoblastic Leukaemia

Acute lymphoblastic leukaemia (ALL) with degeneration of the lymphoid progenitor cells occurs with a frequency of 3.3 per 100,000 children. The median age of onset of the disease is 4.7 years, with a peak between 2 and 5 years of age. Boys are affected 20% more frequently than girls.

The survival rate has steadily improved over recent years. According to the current report by the German Childhood Cancer Registry from 2018, the long-term survival rate after the age of 15 is 90% [1]. Survival rates decrease with increasing age. In adult ALL patients aged <55 years, the survival rates are 50–70% [2].

Both children and adults are often treated according to study protocols. In Germany, these protocols and recommendations are from the multicentre leukaemia study groups (www.kompetenznetz-leukaemie.de).

M. von Wolff (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

N. Gökbuget
Department of Medicine II, Haematology/Oncology, Goethe University, University Hospital, Frankfurt, Germany
e-mail: Goekbuget@em.uni-frankfurt.de

A. Jarisch
Division of Stem Cell Transplantation and Immunology, Department for Children and Adolescents, University Hospital, Goethe University, Frankfurt am Main, Germany
e-mail: Andrea.Jarisch@kgu.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_8

Acute Myeloid Leukaemia

Acute myeloid leukaemia (AML) with a degeneration of the myeloid progenitor cells, i.e. the progenitor cells of the granulocytes and rarely also the thrombocytes and the erythrocytes is, with an incidence of 0.7 per 100,000 children aged <15 years, significantly rarer than ALL and accounts for approx. 20% of childhood leukaemia. The median age of disease onset is 4.1 years, with a low peak in the first 2 years of life and from the age of 13. The ratio of boys to girls is 1.1:1 [1]. The 5-year survival rate is between 60% and 75% (1.3).

AML is the most common form of acute leukaemia in adults. The incidence rises with increasing age to 12.2 cases per 100,000 aged >65 years. Despite improvements in the chances of recovery in younger patients and in prognostically favourable subgroups, the prognosis in older patients remains unfavourable [4].

Treatment Concepts

General Information

Chemotherapy consists of several cycles that last for a few weeks to months. The goal of induction therapy is complete remission, i.e. complete regression of diseased, altered cells in the blood. This includes the elimination of minimal residual disease (MRD), which can be measured with sensitive methods in the bone marrow and/or peripheral blood and represents a relevant prognostic factor.

The initial intensive induction therapy is carried out in hospital as an in-patient and is usually followed by a 1-year consolidation therapy. For an optimal course of therapy, it is important that the period of time between the chemotherapy cycles is as short as possible.

The choice of appropriate consolidation therapy depends on the patient's general condition and the risk of relapse. In general, there are three options for consolidation: further intensive chemotherapy, rarely transplantation with autologous stem cells or allogeneic stem cell transplantation. Stem cell transplantation is an important alternative to chemotherapy, especially for patients with genetic changes or other characteristics that can increase the risk of relapse.

Acute Lymphoblastic Leukaemia

Chemotherapy to treat ALL should be started quickly after diagnosis and is carried out risk-adapted with tested treatment elements. The intensity of the treatment depends on the initially diagnosed genetic markers and the MRD [5] measured at defined times during treatment (day 33, 76 and 96 in children's ALL or before and

after consolidation in adult ALL). How intensive, and thus also how gonadotoxic the therapy will be, is therefore only decided during the course of the first weeks and possibly only months after the start of treatment.

Allogeneic stem cell transplantation during the first remission is only carried out in a subgroup of patients with unfavourable prognostic factors, e.g. in patients with inadequate response to treatment. Depending on the study protocol, the probability of stem cell transplantation is 4–5% [6] for children's ALL and up to 50% for adults. For patients in first remission and with a clearly increased risk of recurrence, allogeneic stem cell transplantation is indicated if there is a matched sibling donor (HLA), or if there is a well-matched related or unrelated donor (matched donor). Paediatric patients with relapsed ALL and an unfavourable risk profile or an inadequate response to re-induction therapy (defined by the MRD level) also have an indication for allogeneic stem cell transplantation [6, 7]. In adult ALL, there is always an indication for bone marrow transplantation after a relapse.

Cranial radiotherapy is only intended for a very small group of paediatric patients whose indication for radiotherapy is defined in the respective treatment protocol. It occurs in 12–16% of cases [8]. In adult ALL, cranial radiotherapy is currently still the standard procedure for the prevention of relapses in the central nervous system (CNS).

Acute Myeloid Leukaemia

Polychemotherapy consists of various therapeutic elements administered over a period of 4–6 months. Cranial radiotherapy is no longer performed in CNS-negative paediatric patients [9]. The indication for allogeneic stem cell transplantation during first remission only exists in patients with unfavourable prognosis or inadequate treatment response [10] and is reported in 6–9% of cases [3]. As with ALL, stem cell transplantation is more common in adults.

Gonadotoxicity of the Treatments

Acute Lymphoblastic Leukaemia

Treatment of ALL consists of four different elements, induction therapy with subsequent induction consolidation, an extra compartment therapy, followed by reinduction therapy (Table 1). In principle, the therapeutic procedure is comparable to adult ALL, whereby individual therapeutic elements differ in time and the treatment is continued after reinduction therapy with further consolidation blocks. Individual substances are administered both as low-dose i.v. injections and as high-dose continuous infusions. After the intensive chemotherapy phase, maintenance therapy is

Table 1 Important cytotoxic agents in ALL treatment (modified according to [7] in children and adults)

Treatment element	Cytotoxic agent (choice)
I. Induction therapy (5–9 weeks) with subsequent induction consolidation (4–12 weeks)	Prednisone or dexamethasone, vincristine, daunorubicin, asparaginase, cyclophosphamide, cytarabine, 6-mercatopurine, etoposide, thioguanine
IIa. Extra compartment therapy (4–8 weeks)	6-mercaptopurine, methotrexate
IIb. Consolidation therapy in adults (4–10 weeks)	Methotrexate, cytarabine, etoposide, vindesine, dexamethasone, asparaginase, 6-mercaptopurine
III. Reinduction therapy (7 weeks)	Dexamethasone or prednisone, asparaginase, doxorubicin, vincristine, cytarabine, cyclophosphamide, thioguanine
IV. Maintenance therapy	6-Mercatopurine, methotrexate

The terminology and treatment durations in this table refer to German study groups. The ALL-BFM studies (Berlin–Frankfurt–Münster study group on the treatment of children and adolescents with ALL), CoALL (Treatment protocol of the Society for Paediatric Oncology and Haematology for the treatment of children with ALL) and GMALL German Multicentre Study Group for adult ALL

given for 2–2.5 years after diagnosis. The individual components of the various treatment elements are listed in Table 1.

Acute Myeloid Leukaemia

Induction therapy consists of an intensive cytotoxic treatment, which leads to long-lasting aplasia. Usually, three cytotoxic drugs are combined: an anthracycline (idarubicin, liposomal daunorubicin or daunorubicin), cytarabine (ARA-C) and etoposide in childhood AML, if necessary. The other three to four chemotherapy cycles are of similar intensity, and the same cytotoxic drugs are usually used. An intensification with one or more high-dose cytarabine cycles is considered standard in paediatric AML and may also be used in adult AML. The importance of maintenance therapy is internationally controversial. In paediatric AML-BFM studies, maintenance therapy for up to a total duration of 1.5 years is given with the drugs 6-thioguanine (daily) and cytarabine (every 4 weeks).

The risk of infertility after ALL or AML treatment depends largely on the need for allogeneic stem cell transplantation (Table 2).

Patients treated with a conventional treatment protocol without stem cell transplantation have a low risk of reduced fertility, which strictly speaking does not require fertility-preservation therapy [11]. However, since it is unclear whether a highly gonadotoxic treatment will follow, cryopreservation of sperm is recommended for men, as this is easy to perform and cryopreservation after chemotherapy is only possible again at intervals of approx. 6 months. Some centres also recommend the administration of GnRH agonists, despite the low toxicity of the first chemotherapy.

Table 2 Gonadotoxicity of various chemotherapy elements for the treatment of ALL and AML

Risk if infertility	Treatment concept
Low risk (<20%)	AML-typical treatment (anthracycline/cytarabine) ALL-typical treatment (multi-agent)
High risk (>80%)	Myeloablative conditioning protocols with high dose cyclophosphamide, busulfan, melphalan
High risk (>90%)	Total body irradiation, TBI: 12 Gy

Patients with an indication for allogeneic stem cell transplantation have more than a 90% risk of infertility [12–14].

Irradiation of the central nervous system as part of ALL and AML treatment can lead to a treatable impairment of the hypothalamic-pituitary axis, particularly in paediatric patients, depending on the dose.

The importance of new treatments such as monoclonal and bispecific antibodies, tyrosine kinase inhibitors and proteasome inhibitors on fertility has not yet been adequately investigated in studies [11].

Probability of Malignant Cells in the Gonads

In untreated leukaemia, malignant cells are always found in the gonads. Even in a remission, it cannot be assumed with certainty that they are free of malignant cells.

Several small studies have investigated whether malignant cells can be found in cryopreserved ovarian tissue (Table 3).

Effectiveness and Risks of Fertility Preservation

The effectiveness of sperm cryopreservation is high. Their later use leads to the birth of a child in about 50% of couples (see chapter “Infertility treatment after fertility preservation therapies”).

After the subsequent transplantation of cryopreserved ovarian tissue, approximately every third woman gives birth (see chapter “Infertility treatment after fertilty preservation therapies”).

In leukaemia, however, it should be remembered that ovarian tissue is preserved only after induction chemotherapy. In principle, it is also possible to preserve ovarian tissue after chemotherapy and transplant it later. For example, Poirot et al. [24] transplanted ovarian tissue from 22 women who had already received (usually milder) chemotherapy before the tissue withdrawal. Alkylating agents were administered to 20 women. The majority of these women had been diagnosed with lymphoma. After initial chemotherapy, e.g. according to the ABVD scheme, a stem cell

transplant was performed with prior cryopreservation of ovarian tissue. After ovarian tissue transplantation, only the duration of tissue activity was significantly reduced compared to women with cryopreservation without prior chemotherapy; all other parameters including pregnancy rates did not differ.

Meirow et al. [25] published a series of ten women in whom ovarian tissue had also been removed after initial chemotherapy and later transplanted. In this study, the pregnancy rates did not differ from a control group without prior chemotherapy.

The first birth after the transplantation of ovarian tissue has also been described in a leukaemia patient [26]. In this 19-year-old, cryopreservation of ovarian tissue was carried out after complete remission of AML. After extensive molecular biological tests, the presence of malignant cells in the ovarian tissue could in all probability be excluded. Recurrence of leukaemia did not occur after transplantation.

It has now been proven that pregnancy after cryopreservation and transplantation of ovarian tissue is possible in leukaemia patients. Nevertheless, the risks of leukaemia retransmission from leukaemia cells in the transplant are so high (Table 3) [27] that cryopreservation of ovarian tissue should only be carried out very cautiously and is considered highly experimental.

Table 3 Detection of malignant cells in ovarian tissue according to various studies (modified according to [15])

Study	Disease[a]	Number of tissue samples examined	Detection of malignant cells by histology and immuno-histochemistry	Detection of molecular markers that allow a PCR study	Detection of malignant cells by PCR
Meirow et al. [16]	CML	2	0/2	2/2	1/2
Dolmans et al. [17]	ALL	18	0/18	16/18	9/16
Rosendahl et al. [18]	ALL, AML, CML	25	0/25	8/25	6/8
Courbiere et al. [19]	CML	1	0/1	1/1	1/1
Greve et al. [20]	ALL, AML, CML	25	—	7/25	4/7
Dolmans et al. [21]	ALL, AML, CML	45	3/45	—	—
Zver et al. [22]	ALL, AML	11	—	2/11	0/2
Soares et al. [23]	ALL, AML, CML	12	1/12	9/12	6/9
Shapira et al. [15]	AML	1	0/1	0/1	0/0

[a]*AML* Acute myeloid leukaemia, *ALL* acute lymphoblastic leukaemia, *CML* chronic myeloid leukaemia

Practical Approach

The difficulties for defining the indication for fertility-preservation therapy in acute leukaemia are:

1. In acute leukaemia, induction chemotherapy (with little gonadotoxicity) must usually begin immediately, therefore fertility-preservation therapy cannot be started except for sperm collection and the administration of GnRH agonists.
2. After the start of induction chemotherapy, no oocytes and sperm can be preserved within ca. 6 months after chemotherapy.
3. It is often unclear before the start of chemotherapy, and directly after induction chemotherapy, whether a highly gonadotoxic bone marrow transplantation will be required, as this decision depends on the response of the leukaemia during treatment.
4. If a bone marrow transplant is performed, even if there is no evidence of neoplastic cells in the blood or bone marrow, it must be assumed that malignant cells are still present in the gonadal tissue. Thus, the tissue can probably only be used in at least 10 years if it is possible to develop suitable techniques (see chapter "Further Fertility Preservation Techniques").

The following practical procedures can be derived from the above:

- If acute leukaemia is diagnosed, the man should have his sperm cryopreserved before starting induction chemotherapy. In women, fertility-preservation therapy is not possible because the available time is too short. However, despite the low gonadal toxicity, GnRH agonists can be considered.
- Should it become apparent that bone marrow transplantation is required as consolidation therapy, cryopreservation of ovarian (see chapters "Removal of Ovarian Tissue" and "Transportation, Cryopreservation and Storage of Ovarian Tissue") or testicular tissue (if semen collection was not possible, see chapter "Cryopreservation of Sperm and Testicular Tissue") may be considered. However, this only makes sense if the tissue is to be used after about 10 years at the earliest, since the techniques required to use the tissue (e.g. isolation of the follicles and isolation of testicular stem cells) have not yet been sufficiently established (see chapter "Further Fertility Preservation Techniques"). The patient must also be made aware that it may never be possible to use testicular stem cells and that the doctor may refuse to transplant the tissue.
- Before starting bone marrow transplantation, women may receive GnRH agonists (see chapter "GnRH Agonists") to reduce gonadal toxicity and the risk of severe uterine bleeding. However, there is no data on whether GnRH agonists can actually reduce the risk of premature ovarian failure during bone marrow transplantation. However, it has been shown that the risk of bleeding is lower among GnRH agonists compared to progestogens [28] (see Fig. 1).

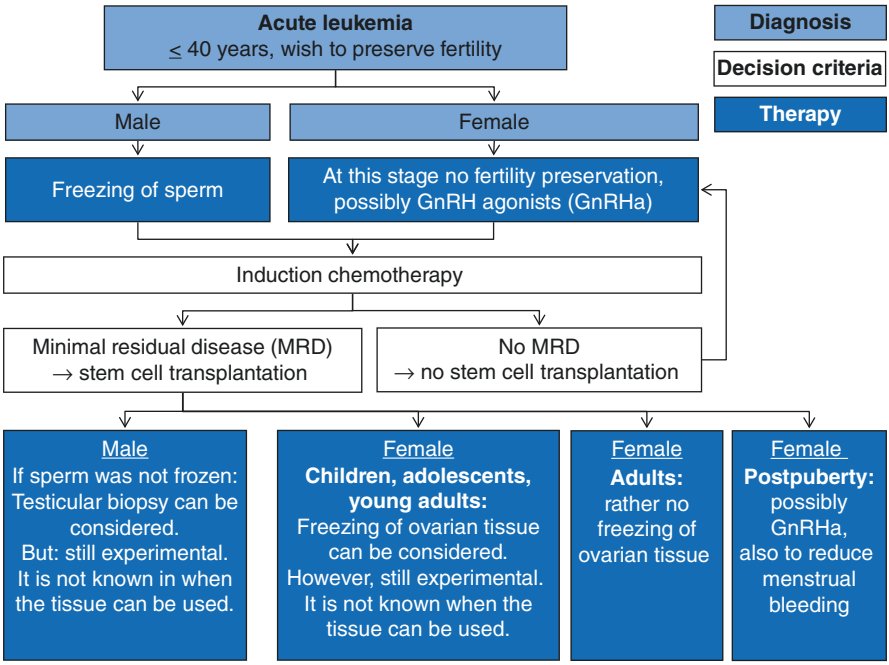


Fig. 1 Algorithm for fertility preservation in acute leukaemia

References

1. Kaatsch P, Grabow D, Spix C. German Childhood Cancer Registry – Annual Report 2018 (1980-2017) Institute of Medical Biostatistics, Epidemiology and Informatics (MBEI) at the University Medical Center of the Johannes Gutenberg University Mainz, 2019., http://www.kinderkrebsregister.de/typo3temp/secure_downloads/22605/0/2df4719687ba2596d4216218a4f4632763b64847/jb2018s.pdf.
2. Bassan R, Bourquin JP, DeAngelo DJ, Chiaretti S. New approaches to the management of adult acute lymphoblastic leukemia. J Clin Oncol. 2018:JCO2017773648. https://doi.org/10.1200/JCO.2017.77.
3. Rasche M, Zimmermann M, Borschel L, Bourquin JP, Dworzak M, Klingebiel T, et al. Successes and challenges in the treatment of pediatric acute myeloid leukemia: a retrospective analysis of the AML-BFM trials from 1987 to 2012. Leukemia. 2018;32:2167–77.
4. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129:424–47.
5. Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer R, Möricke A, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood. 2010;115:3206–14.
6. Sureda A, Bader P, Cesaro S, Dreger P, Duarte RF, Dufour C, et al. Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2015. Bone Marrow Transplant. 2015;50:1037–56.

7. AWMF 2016 S1-Leitlinie 025/014: Akute lymphoblastische- (ALL) Leukämie im Kindesalter, Stand 04/2016, https://www.awmf.org/uploads/tx_szleitlinien/025-014l_S1_Akute_lymphoblastische_Leukaemie_ALL_2016-04.pdf.
8. Vora A, Andreano A, Pui CH, Hunger SP, Schrappe M, Moericke A, et al. Influence of cranial radiotherapy on outcome in children with acute lymphoblastic leukemia treated with contemporary therapy. J Clin Oncol. 2016;34:919–26.
9. Creutzig U, van den Heuvel-Eibrink MM, Gibson B, Dworzak MN, Adachi S, de Bont E, et al. Committee of the International BFM Study Group. Diagnosis and management of acute myeloid leukemia in children and adolescents: recommendations from an international expert panel. Blood. 2012;120:3187–205.
10. AWMF 2019 S1-Leitlinie 025–031 - Akute myeloische Leukämie (AML) im Kindes- und Jugendalter, Stand 03/2019, https://www.awmf.org/uploads/tx_szleitlinien/025-031l_S1_Akute-myeloische-Leukaemie%E2%80%93AML%E2%80%93Kinder-Jugendliche_2019-09.pdf.
11. Salama M, Anazodo A, Woodruff TK. Preserving fertility in female patients with hematological malignancies: a multidisciplinary oncofertility approach. Ann Oncol. 2019;30:1760–75.
12. Borgmann-Staudt A, Rendtorff R, Reinmuth S, Hohmann C, Keil T, Schuster FR, et al. Fertility after allogeneic haematopoietic stem cell transplantation in childhood and adolescence. Bone Marrow Transplant. 2012;47:271–6.
13. Pfitzer C, Orawa H, Balcerek M, Langer T, Dirksen U, Keslova P, et al. Dynamics of fertility impairment and recovery after allogeneic haematopoietic stem cell transplantation in childhood and adolescence: results from a longitudinal study. J Cancer Res Clin Oncol. 2015;141:135–42.
14. Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim HA Jr, Peccatori FA, et al. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med. 2016;14:1.
15. Shapira M, Raanani H, Derech Chaim S, Meirow D. Challenges of fertility preservation in leukemia patients. Minerva Ginecol. 2018;70:456–64.
16. Meirow D, Hardan I, Dor J, Fridman E, Elizur S, Ra'anani H, et al. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. Hum Reprod. 2008;23:1007–13.
17. 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;116:2908–14.
18. Rosendahl M, Andersen MT, Ralfkiær E, Kjeldsen L, Andersen MK, Andersen CY. Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. Fertil Steril. 2010;94:2186–90.
19. Courbiere B, Prebet T, Mozziconacci MJ, Metzler-Guillemain C, Saias-Magnan J, Gamerre M. Tumor cell contamination in ovarian tissue cryopreserved before gonadotoxic treatment: should we systematically exclude ovarian autograft in a cancer survivor? Bone Marrow Transplant. 2010;45:1247–8.
20. Greve T, Clasen-Linde E, Andersen MT, Andersen MK, Sørensen SD, Rosendahl M, et al. Cryopreserved ovarian cortex from patients with leukemia in complete remission contains no apparent viable malignant cells. Blood. 2012;120:4311–6.
21. Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, et al. A review of 15 years of ovarian tissue bank activities. J Assist Reprod Genet. 2013;30:305–14.
22. Zver T, Alvergnas-Vieille M, Garnache-Ottou F, Roux C, Amiot C. A new method for evaluating the risk of transferring leukemic cells with transplanted cryopreserved ovarian tissue. J Assist Reprod Genet. 2015;32:1263–6.
23. Soares M, Saussoy P, Maskens M, Reul H, Amorim CA, Donnez J, et al. Eliminating malignant cells from cryopreserved ovarian tissue is possible in leukaemia patients. Br J Haematol. 2017;178:231–9.
24. Poirot C, Fortin A, Lacorte JM, Akakpo JP, Genestie C, Vernant JP, et al. CAROLéLISA Cooperative Group. Impact of cancer chemotherapy before ovarian cortex cryopreservation on ovarian tissue transplantation. Hum Reprod. 2019;34:1083–94.

25. Meirow D, Ra'anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertil Steril. 2016;106:467–74.
26. Shapira M, Raanani H, Barshack I, Amariglio N, Derech-Haim S, Marciano MN, et al. First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination. Fertil Steril. 2018;109:48–53.
27. Gook D, Hale L, Polyokov A, Stern K. Outcomes from heterotopic and orthotopic grafting of human cryopreserved ovarian tissue. Hum Reprod. 2019;34(Suppl 1):i60–1.
28. Meirow D, Rabinovici J, Katz D, Or R, Shufaro Y, Ben-Yehuda D. Prevention of severe menorrhagia in oncology patients with treatment-induced thrombocytopenia by luteinizing hormone-releasing hormone agonist and depo-medroxyprogesterone acetate. Cancer. 2006;107:1634–41.

Ovarian Tumours and Ovarian Cancer

Maren Goeckenjan, Pauline Wimberger, and Michael von Wolff

Ovarian Tumours in Young Women and Impact on Fertility

In the *Ferti*PROTEKT network, around 1.5% of all consultations on fertility preservation between 2007 and 2013 were initiated after the diagnosis of an ovarian tumour [1].

Surgery for *benign ovarian tumours* nowadays respects the individual importance of a woman's fertility, and consistently protects the surrounding ovarian tissue if possible. In chronic diseases with benign changes to the ovaries, e.g. endometriosis, it has only been acknowledged by medical professionals in recent decades how important it is to preserve the surrounding ovarian tissue while sparing the primordial and primary follicles in young women.

With *malignant ovarian tumours*, the individual decision regarding the surgical and oncological procedure is more difficult in young women who wish to have children. Ovarian tumours are of different biological origins and exhibit different biological behaviour.

M. Goeckenjan (✉) · P. Wimberger
TU Dresden, University Hospital, Dresden, Germany
e-mail: maren.goeckenjan@uniklinikum-dresden.de; pauline.wimberger@uniklinikum-dresden.de

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_9

Stage-Dependent Prognosis

Borderline Ovarian Tumours

About 10–20% of all malignant diseases of the ovary are borderline ovarian tumours (BOT). The diagnosis is usually made in the early stages [2]. BOT has a very good prognosis compared to ovarian cancer, with 5-year survival rates of >95% in all stages. One-third of affected patients are younger than 40 years of age and are therefore directly affected by the impairment of the ovarian reserve due to illness and treatment. The detection of invasive peritoneal implants is the most important prognostic factor in BOT [3].

Epithelial Ovarian Cancer

Epithelial ovarian carcinoma is more common in older women, but also occurs in women of reproductive age. It represents the largest group of malignant ovarian tumours in young women (Table 1). In contrast to other gynaecological tumours, effective early detection is still not possible. Due to diagnosis in the higher disease stage, the 5-year survival rate is low and was 41% in 2013–2014. In stage FIGO I, however, the prognosis is excellent at over 90% [4].

The most important prognostic factors for ovarian cancer are the post-operative tumour residue and the tumour stage. Other established prognostic factors – apart from age and general health – are tumour grading, histological type and guideline-based treatment [5].

Malignant Germ Cell Tumours and Malignant Sex Cord Stromal Tumours

Malignant germ cell tumours and malignant sex cord stromal tumours account for up to 10% of malignant ovarian tumours [6]. They particularly affect girls and young women. These malignant ovarian tumours have a much better prognosis than epithelial ovarian carcinoma [7].

Effects of Oncological Treatment on Fertility

Version 3.0 of the German, Austrian and Swiss AWMF-S3 guideline on malignant ovarian tumours provides the current consensus-based recommendations for oncological treatment [5]. In principle, both the biology of the disease and the individual

Table 1 Characteristics of different ovarian tumours and ovarian cancer, 5-year survival rate and oncological treatment [3–7]

Ovarian tumour	Characteristics of the disease	Relative 5-year survival rate	Oncological treatment
Benign ovarian tumours	Ovarian tumours with different biological origin: Teratoma, endometrioma, mucinous or serous cystoma	No impact on lifetime	Excision of the tumour with preservation of functional tissue of the ovary
Borderline ovarian tumours (BOT)	Mean age at diagnosis: 45 years, Incidence: 1.8–4.8/100,000 women, 7–30% with bilateral occurrence at diagnosis	FIGO stage I: 95–97% FIGO stage III: 65–87%	Fertility-sparing surgery with surgical staging in early stages. In later stages surgical staging with bilateral salpingo-oophorectomy, omentectomy, multiple peritoneal biopsies with complete macroscopic resection. Only surgical treatment, no chemotherapy necessary.
Malignant germ cell tumours	5% of malignant ovarian tumours. Diagnosis prior to age of 30 yrs.: 75% of diagnosis in FIGO stage I. High sensibility for chemotherapy, mostly unilateral occurrence	85–94%	Fertility-sparing surgery possible. Surgical staging. Chemotherapy indicated in stages > FIGO IA, three cycles of platinum-based combination (PEB).
Malignant sex cord stromal tumours	5% of malignant ovarian tumours	Overall good prognosis, >95%	Fertility-sparing surgery possible. Surgical staging, hysteroscopy and curettage if uterus-sparing surgery is performed. Chemotherapy indicated at FIGO IC and higher.
Ovarian carcinoma	Mean age at diagnosis: 70 yrs. Detection in 20% in FIGO stage I, 60% in FIGO stage III; often BRCA-mutation (found in 20%)	43% all stages UICC stage I 89% UICC stage II 77% UICC stage III 41% UICC stage IV 17%	Treatment according to current guidelines: Surgery adequate to stage. Platinum mono-chemotherapy if stage FIGO > IA G1; Platinum–taxane-based chemotherapy if stage FIGO II or higher; FIGO IIIB and later stages: Platinum and taxane-based chemotherapy plus bevacizumab; BRCA 1/2-mutation and FIGO III/IV: Platinum–taxane combination, followed by maintenance therapy with a PARP inhibitor.

stage must be taken into account when choosing fertility-preservation measures in patients with malignant ovarian tumours. The German, Austrian and Swiss AWMF-S2k guideline on fertility preservation in oncological disease refers to the AWMF-S3 guideline [8] with regard to malignant ovarian tumours.

A recent retrospective study from Italy with 548 women with malignant ovarian tumours after fertility preservation and a follow-up period of about 15 years showed normal fertility rates of almost 90% [9] if they wished to conceive after their illness.

Borderline Ovarian Tumours

Despite the very good prognosis of borderline ovarian tumours in young women, the recommended surgical procedure basically includes the initial complete removal of the tumour with salpingo-oophorectomy and adequate surgical staging, since the post-operative tumour residue is the most important prognostic factor. The primary goal of surgical treatment is – as for ovarian cancer – complete resection. Staging includes inspection of the abdomen with peritoneal washing cytology, resection of all conspicuous areas and exemplary peritoneal biopsies, also from an inconspicuous peritoneum, as well as omentectomy. If the patient does not wish to have children, a contralateral salpingo-oophorectomy should also be performed.

If fertility preservation is desired, conservation of the contralateral ovary or, in the case of bilateral involvement, of part of the ovary and the uterus is justifiable [10]. The AWMF-S3 guideline on malignant ovarian tumours [5] emphasises the necessity of staging in accordance with the guidelines in the presence of a BOT and the need for clarification on the increased risk of recurrence with fertility-sparing surgery.

Epithelial Ovarian Cancer

The goal of surgical treatment of ovarian cancer is the macroscopically complete tumour resection. Surgical treatment with longitudinal laparotomy according to guidelines includes bilateral salpingo-oophorectomy, hysterectomy, omentectomy, appendectomy in cases with invasive mucinous subtype or infiltration, peritoneal biopsy, and in early stages or in cases of infiltration, bilateral pelvic and paraaortic lymphonodectomy extending to the renal hilum.

A fertility-preserving surgical procedure can only be chosen under certain conditions for a low-grade and unilateral tumour stage FIGO I if the patient wishes to have children after adequate staging.

No adjuvant chemotherapy is indicated in patients with FIGO IA, G1. In patients with FIGO IA G2, IB G1–2, chemotherapy with carboplatin mono can be administered over six cycles (Table 2). From FIGO stage IC or IA/B G3, women should receive platinum-containing chemotherapy, from FIGO IIB with additional

Table 2 Gonadotoxicity of current chemotherapy protocols for malignant ovarian tumours

Chemotherapy protocol	Indication	Rate of chemotherapy-induced amenorrhoea
BEP—Bleomycin, etoposide, cisplatin	Malignant germ cell tumours	95%, persistent in about 30% [14]
Six cycles carboplatin mono	"Might": Starting from stage FIGO IA G2, IB/C G1–2 "Should": FIGO IC, IA/B G3 and higher	Only very limited data on platinum therapy
Six cycles of carboplatin in combination with paclitaxel	Stages starting from FIGO IIB, From FIGO IB G1/2 after individual decision	No studies available

paclitaxel. Additive treatment with the monoclonal antibody bevacizumab should be considered for advanced ovarian carcinoma from FIGO IIIB onwards, as this can significantly extend the progression-free interval [11].

A 70% reduction in the risk of recurrence after 3 years after treatment response to platinum/taxane chemotherapy with maintenance therapy using the PARP inhibitor olaparib was shown in a prospective, randomized, placebo-controlled phase III study with proven BRCA1 or −2 mutation and stage III or IV [12]. New data also show a significantly prolonged progression-free survival in high-grade FIGO III/IV ovarian cancer independent of a BRCA mutation with PARP inhibitors as maintenance therapy [13].

Malignant Germ Cell Tumours

Malignant germ cell tumours are treated, if possible, with fertility-preserving methods such as unilateral salpingo-oophorectomy, omentectomy and peritoneal biopsies. Adjuvant combination chemotherapy, mostly according to the BEP scheme (Table 2), is recommended in patients with residual tumour or from FIGO > IA. Chemotherapy-induced amenorrhoea is reversible in 70%, and studies have described a fairly good prognosis for fertility despite the combined surgical and gonadotoxic reduction in the ovarian reserve [14].

There are no reliable studies on the gonadotoxicity of platinum-containing chemotherapy. However, depending on age, the gonadotoxic effect can be assumed as mid-range compared with other regimes of chemotherapy.

Malignant Sex Cord Stromal Tumours

Hysteroscopy and fractional abrasion are recommended for patients with malignant sex cord stromal tumours undergoing treatment with uterus preservation, as there is an increased risk of endometrial hyperplasia and carcinoma. Otherwise, surgical treatment corresponds with that of malignant germ cell tumours. Adjuvant platinum-containing chemotherapy can be considered from stage FIGO IC and higher as well as for residual tumours.

Effectiveness and Risks of Fertility-Preservation Therapy

Borderline Ovarian Tumours

Preservation of the ovaries and uterus is not associated with a great increase in risk in stage I borderline tumours after sufficient surgical staging, if the prognosis is excellent (Table 3). However, several retrospective observational studies show that there is an increased risk of recurrence, especially with BOT cystectomy. The world‘s largest multicentre retrospective study with 280 young German patients with BOT currently indicates an increased risk of recurrence in the remaining ovarian tissue, although the risks of BOT recurrence and invasive transformation are not reflected by a significant deterioration in survival rates [2]. In the event of a relapse, invasive epithelial ovarian carcinoma is present in up to 30% of cases.

Fertility after BOT and ovarian preservation is limited by the surgically reduced ovarian reserve. However, data on pregnancies are rarely documented in registries

Table 3 Malignant ovarian tumours and possible risks of fertility preservation

Ovarian tumour	Invasive fertility preservation techniques	Risks
Borderline ovarian tumours, BOT	Fertility-sparing surgery with preservation of uterus and ovary/ovaries. After surgery, controlled ovarian stimulation and cryopreservation of oocytes possible.	Overall increase in recurrence discussed, but especially after cystectomy
Ovarian cancer	FIGO IA G1: Fertility sparing surgery possible.	No elevated risk of recurrence. If chemotherapy is necessary fertility-sparing surgery is not possible.
Malignant germ cell tumours	Fertility-sparing surgery GnRH agonist treatment during chemotherapy	Only low risk of recurrence after surgical staging and adequate surgery. No sufficient data if GnRH agonist treatment reduces gonadotoxicity of chemotherapy.

or longitudinal observational studies. A retrospective multicentre study from France showed a fertility rate of 32.3% in 65 women who wanted to have children out of an initial 162 women who had undergone fertility-sparing surgery with BOT [15].

Epithelial Ovarian Cancer

In epithelial ovarian carcinoma, fertility-sparing surgery does not significantly worsen the recurrence-free interval and survival after clinical preselection of the patient [16]. This is shown by the 5-year survival rate in a large American cohort study with 825 women after fertility preservation compared to women with guideline-based surgery [17]. A prerequisite for this is an individual risk assessment and clarification, as well as close follow-up until birth (Table 3). Once family planning has been completed, complementary surgery should be performed.

It is difficult to find data on pregnancies after ovarian cancer and fertility preservation. Only in recent years have oncological patients been followed up consistently and on a long-term basis regarding their fertility [18].

Hormonal Stimulation and Cryopreservation of Oocytes

It should be noted that ovarian surgery in women with ovarian tumours generally reduces the ovarian reserve. This functional loss of ovarian tissue due to previous surgery becomes apparent when ovarian stimulation is used to obtain oocytes. Women with ovarian cancer received an average of 7.3 oocytes compared to 13.3 oocytes in women with other malignancies [19]. The expected effectiveness of ovarian stimulation per stimulation cycle is therefore lower and several stimulation cycles may even be required (see chapter “Ovarian Stimulation to Collect Oocytes”) to achieve a sufficiently high chance of a live birth (see chapter “Cryopreservation of Unfertilized and Fertilized Oocytes”).

Freezing of Oocytes

IVF with controlled ovarian stimulation does not seem to increase the risk of BOT and ovarian cancer in previously healthy women [20]. However, it is not clear whether ovarian stimulation after treatment for BOT slightly increases the risk of relapse [21]. A recent study of 91 women with gynaecological cancer and fertility preservation shows the results for ovarian stimulation and pregnancy rates after cryopreservation of oocytes in an American centre, where 5 out of women were

pregnant after embryo transfer. Ovarian cancer accounted for almost half the diseases [22].

Freezing of Ovarian Tissue

Ovarian cryopreservation is currently not recommended in patients with ovarian cancer. Due to the theoretically significantly increased risk of auto-transplantation of tumour cells that have remained in the removed, cryopreserved and later auto-transplanted tissue, the oncological risk for the patient is considered too high.

Cryopreservation of ovarian tissue may be a therapeutic strategy if, instead of autotransplantation of the tissue, the still experimental techniques for the generation of oocytes by xenotransplantation in other species, in vitro maturation or formation of an artificial ovary (see chapter "Further Fertility Preservation Techniques"), are proven as effective and safe. However, the effectiveness of these experimental techniques cannot be assessed at present.

GnRH Agonists

If chemotherapy is induced in addition to fertility-sparing surgery, GnRH agonists (GnRHa) should be considered for protection of the ovaries. It can be assumed but it has not been proven that GnRHa is just as effective in women with ovarian cancer as in women with breast cancer (see chapters "Breast cancer" and "GnRH Agonists"). Impairment of the effectiveness of chemotherapy by GnRHa in patients with hormone-sensitive tumours is discussed, but is very unlikely [23].

BRCA1/2-Mutation

A particular aspect of counselling women with ovarian cancer who wish to have children is the genetic predisposition in the case of a proven BRCA1/2 mutation. A new recommendation within the consortium of familial breast and ovarian cancer is that all patients with ovarian cancer up to the age of 80 years should receive genetic counselling and BRCA1/2 testing, even without further familial predisposition [24]. According to the German, Austrian, Swiss AWMF guideline "Diagnosis, Treatment and Aftercare of Malignant Ovarian Tumours", women in risk constellations should receive genetic counselling and, if a BRCA1/2 mutation is detected, receive prophylactic bilateral salpingo-oophorectomy after family planning is complete [5]. The individually increased risk of ovary preservation and the risk of passing on the familial predisposition to one's own children should also be addressed during the consultation on fertility preservation.

If women are to be stimulated to cryopreserve oocytes after treatment for ovarian cancer and in the presence of a BRCA mutation, the reduced ovarian reserve from the BRCA mutation (see chapter "Breast cancer") in addition to the above-mentioned restriction of the ovarian reserve by surgery must also be taken into account.

Practical Approach

After careful selection and good risk consultation, fertility-sparing surgery can also be performed on women with malignant ovarian tumours. Fertility-sparing surgery with complete staging only appears to be sensible and oncologically safe for women under 40 years of age with a desire to have children who have unilateral FIGO IA G1 ovarian cancer or borderline ovarian tumours.

For malignant germ cell tumours and malignant sex cord stromal tumours, this should be considered in combination with adjuvant chemotherapy at the appropriate stage. In these women, drug-assisted ovarian protection with GnRHa may be useful in addition to chemotherapy.

All women must be informed that in principle, after the desire for a child has been fulfilled, the completion surgery should be performed.

Figure 1 shows the procedure of fertility-preservation measures for ovarian tumours.

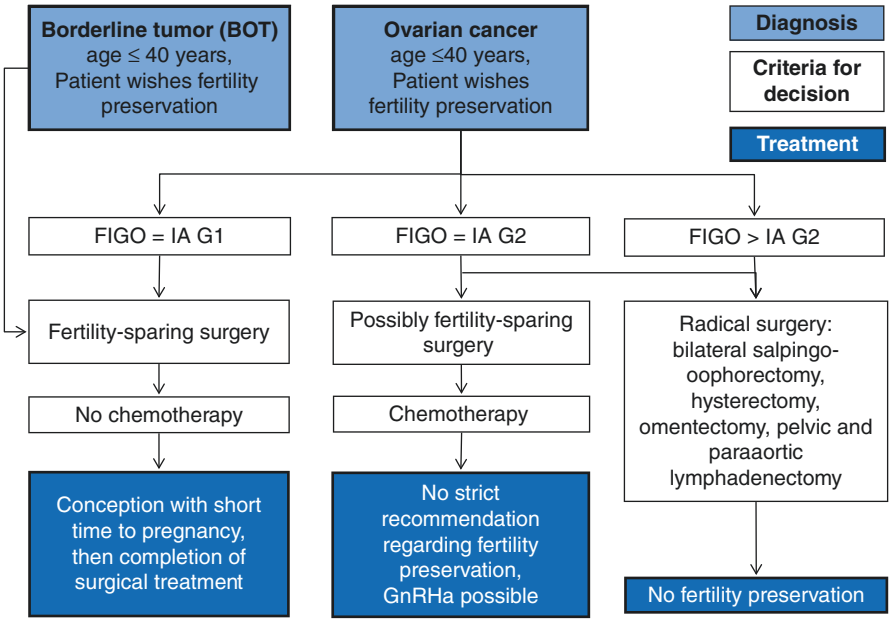


Fig. 1 Algorithm for fertility preservation in patients with borderline ovarian tumours (BOT) and ovarian cancer

References

1. von Woff M, Dittrich R, Liebenthron J, Nawroth F, Schüring AN, Bruckner T, et al. Fertility preservation counselling and treatment for medical reasons: data from a multinational network of over 5000 women. Reprod Biomed Online. 2015;31:605–12. https://doi.org/10.1016/j.rbmo.2015.07.013.
2. Trillsch F, Mahner S, Woelber L, Vettorazzi E, Reuss A, Ewald-Riegler N, et al. Age-dependent differences in borderline ovarian tumours (BOT) regarding clinical characteristics and outcome: results from a sub-analysis of the Arbeitsgemeinschaft Gynaekologische Onkologie (AGO) ROBOT study. Ann Oncol. 2014;25:1320–7. https://doi.org/10.1093/annonc/mdu119.
3. Seong SJ, Kim DH, Kim MK, Song T. Controversies in borderline ovarian tumors. J Gynecol Oncol. 2015;26:343–9. https://doi.org/10.3802/jgo.2015.26.4.343.
4. https://www.krebsdaten.de/Krebs/DE/Content/Publikationen/Krebs_in_Deutschland/kid_2019/kid_2019_c56_eierstoecke.pdf?__blob=publicationFile, last retrieval 25.01.2020.
5. S3-Leitlinie. Diagnostik, Therapie und Nachsorge maligner Ovarialtumoren, Langversion 3.0 2019, AWMF-Registernummer: 032/035OL. https://www.leitlinienprogramm-onkologie.de/fileadmin/user_upload/Downloads/Leitlinien/Ovarialkarzinom/Version_4/LL_Ovarialkarzinom_Langversion_4.01.pdf, last retrieval 26.01.2020.
6. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med. 2017;14:9–32. https://doi.org/10.20892/j.issn.2095-3941.2016.0084.
7. Park JY, Kim DY, Suh DS, Kim JH, Kim YM, et al. Outcomes of pediatric and adolescent girls with malignant ovarian germ cell tumors. Gynecol Oncol. 2015;137:418–22. https://doi.org/10.1016/j.ygyno.2015.03.054.
8. Deutsche Gesellschaft für Gynäkologie und Geburtshilfe (DGGG), Deutsche Gesellschaft für Reproduktionsmedizin (DGRM), Deutsche Gesellschaft für Urologie (DGU). Leitlinie: Fertilitätserhaltung bei onkologischen Therapien. Level S2k, AWMF Register Nr. 015/082, November 2017. http://www.awmf.org/leitlinien/detail/ll/015-082.html. Last retrieval 26.01.2020.
9. Ceppi L, Galli F, Lamanna M, Magni S, Dell'Orto F, Verri D, et al. Ovarian function, fertility, and menopause occurence after fertility-sparing surgery and chemotherapy for ovarian neoplasms. Gynecol Oncol. 2019;152:346–52. https://doi.org/10.1016/j.ygyno.2018.11.032.
10. Darai E, Fauvet R, Uzan C, Gouy S, Duvillard P, Morice P. Fertility and borderline ovarian tumor: a systematic review of conservative management, risk of recurrence and alternative options. Hum Reprod Update. 2013;19:151–66. https://doi.org/10.1093/annonc/mdt430.
11. Meinhold-Heerlein I, Fotopoulou C, Harter P, Kurzede C, Mustea A, Wimberger P, et al. The new WHO classification of ovarian, fallopian tube, and primary peritoneal cancer and its clinical implications. Arch Gynecol Obstet. 2016;293:695–700. https://doi.org/10.1007/s00404-016-4035-8.
12. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2018;379:2495–505. https://doi.org/10.1056/NEJMoa1810858.
13. Ray-Coquard I, Pautier P, Pignata S, Perol D, Gonzales-Martin A, Berger R, PAOLA-1 Investigators. Olaparid plus Bevacizumab as first-line maintenance in ovarian cancer. N Engl J Med. 2019;381:2416–28. https://doi.org/10.1056/NEJMoa1911361.
14. Gadducci A, Lanfredini N, Tana R. Menstrual function and childbearing potential after fertility-sparing surgery and platinum-based chemotherapy for malignant ovarian germ cell tumours. Gynecol Endocrinol. 2014;30:467–71. https://doi.org/10.3109/09513590.2014.907262.
15. Fauvet R, Poncelet C, Boccara J, Descamps P, Fondrinier E, Darai E. Fertility after conservative treatment for borderline ovarian tumors: a French multicenter study. Fertil Steril. 2005;83:284–90. https://doi.org/10.1016/j.fertnstert.2004.10.009.
16. Wright JD, Shah M, Mathew L, Burke WM, Culhane J, Goldman N, Schiff PB, Herzog TJ. Fertility preservation in young women with epithelial ovarian cancer. Cancer. 2009;115:4118–26. https://doi.org/10.1002/cncr.24461.

17. Melamed A, Rizzo AE, Nitecki R, Gockley AA, Bregar AJ, Schorge JO, et al. All-cause mortality after fertility-sparing surgery for stage I epithelial ovarian cancer. Obstet Gynecol. 2017;130:71–9. https://doi.org/10.1097/AOG.0000000000002102.
18. Massarotti C, Scaruffi P, Lambertini M, Sozzi F, Remorgida V, Anserini P. Beyond fertility preservation: role of the oncofertility unit in the reproductive and gynecological follow-up of young cancer patients. Hum Reprod. 2019;34:1462–9. https://doi.org/10.1093/humrep/dez108.
19. Von Wolff M, Bruckner T, Strowitzki T, Germeyer A. Fertility preservation: ovarian response to freeze oocytes is not affected by different malignant diseases-an analysis of 992 stimulations. J Assist Reprod Genet. 2018;35:1713–9. https://doi.org/10.1007/s10815-018-1227-0.
20. Siristatidis C, Sergentanis TN, Kanavidis P, Trivella M, Sotiraki M, Mayromatis I, et al. Controlled ovarian hyperstimulation for IVF: impact on ovarian, endometrial and cervical cancer—a systematic review and meta-analysis. Hum Reprod Update. 2013;19:105–23. https://doi.org/10.1093/humupd/dms051.
21. Denschlag D, von Wolff M, Amant F, Kesic V, Reed N, Schneider A, et al. Clinical recommendation on fertility preservation in borderline ovarian neoplasm: ovarian stimulation and oocyte retrieval after conservative surgery. Gynecol Obstet Investig. 2010;70:160–5. https://doi.org/10.1159/000316264.
22. Akel RA, Guo XM, Moravek MB, Confino R, Smith KN, et al. Ovarian stimulation is safe and effective for patients with gynecologic cancer. J Adolesc Young Adult Oncol. 2020; https://doi.org/10.1089/jayao.2019.0124.
23. Lin QY, Wang YV, Wenig HN, Sheng XJ, Jiang QP, Yang ZY. Influence of gonadotropin-releasing hormone agonist on the effect of chemotherapy upon ovarian cancer and the prevention of chemotherapy-induced ovarian damage. J Zhejiang Univ Sci B. 2012;13:894–903. https://doi.org/10.1631/jzus.B1100369.
24. Hauke J, Hahnen E, Schneider S, Reuss A, Richters L, Kommoss S, et al. Deleterious somatic variants in 473 consecutive individuals with ovarian cancer: results of the observational AGO-TR1 study (NCT02222883). J Med Genet. 2019;56:574–80. https://doi.org/10.1136/jmedgenet-2018-105930.

Cervical Cancer

Maren Goeckenjan, Pauline Wimberger, and Michael von Wolff

Epidemiology of Cervical Cancer in Young Women and Impact on Fertility

To date, cervical carcinoma is the second most common cancer in women worldwide. In the *Ferti*PROTEKT network, approximately 1.5% of consultations were due to cervical cancer [1]. Approximately one in four women worldwide are under 35 years of age at the time of initial diagnosis [2]. However, the incidence of cervical carcinoma has been steadily declining for decades in developed countries following the introduction of statutory early detection programmes. At the same time, malignant changes of the cervix in earlier stages are detected malginant changes of the cervix are detected earlier: One-third of all cervical cancers in developed countries are currently diagnosed at FIGO stage I [3].

Human papillomavirus (HPV) vaccination reduces the risk of cervical cancer in situ and cervical cancer [4]. However, vaccine efficacy is mainly depending on the vaccination coverage in the population.

In approx. 80% of cases, cervical cancer is a squamous cell carcinoma. However, adenocarcinoma, the histological subtype with a worse prognosis, affects young women in particular and is less likely to be detected by early screening [5].

M. Goeckenjan (✉) · P. Wimberger
TU Dresden, University Hospital, Dresden, Germany
e-mail: maren.goeckenjan@uniklinikum-dresden.de; pauline.wimberger@uniklinikum-dresden.de

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_10

Stage-Dependent Prognosis

The tumour stage is one of the most important parameters for estimating prognosis in cervical cancer. It has a very good prognosis in the early stages (Table 1). Other clear prognostic and risk factors for cervical cancer are lymph node involvement, lymphatic-, neoplastic vascular invasion, tumour grading, histological subtype and the resection margins. Patients without lymph node involvement have a 5-year survival rate of 90%, while proven pelvic lymph node involvement reduces the rate to 20–60%, depending on the location [5].

Influence of Treatments on Fertility

Cervical Carcinoma In Situ

Cervical carcinoma in situ does not limit fertility per se. Treatment should be performed in the way that is therapeutically appropriate. Adaptation of the cone and the technique, optimally with a large loop excision of the transformation zone (LLETZ), reduces the negative effect on the occlusive function of the cervix in subsequent pregnancies and thus in particular the risk of premature birth [8].

Cervical Cancer

Oncological treatment for cervical carcinoma can influence female fertility in many ways (Fig. 1).

Table 1 Characteristics and stage-dependent prognosis of cervical cancer, data from German cancer registries [6, 7]

	Diagnosis of cervical cancer in 2016 (*n*)	Median age at diagnosis (cervical cancer)	Relative 5 years survival rates (FIGO 2009)	Median age at diagnosis (carcinoma in situ)
Cervical cancer	4380	55 years	FIGO stage I: 94% FIGO stage II: 63% FIGO stage III: 54% FIGO stage IV: 23% All stages: 67%	34 years

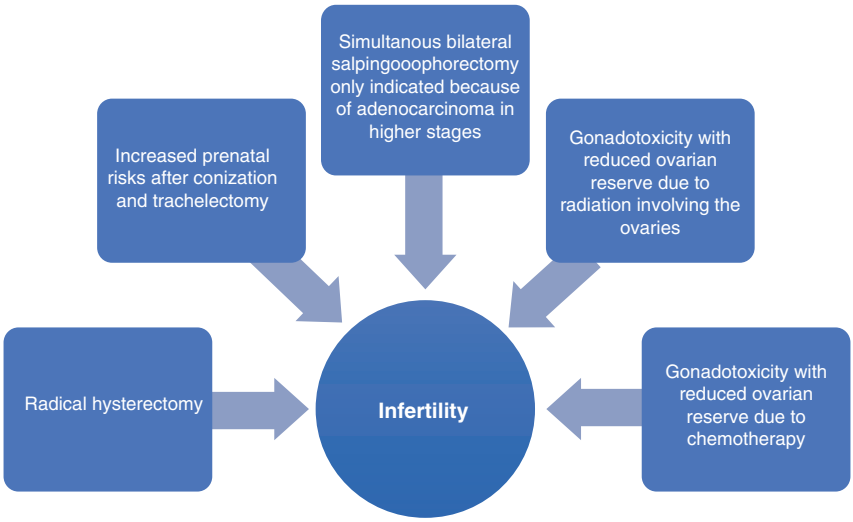


Fig. 1 Impairment of fertility as a result of cervical cancer and oncological treatment

Surgery

In early stages of cervical cancer, surgery is primarily indicated as a curative treatment and is equally effective as primary combined radiochemotherapy. Cervical treatment and therefore fertility-preserving therapy can only be permitted in early tumour stages up to a maximum of FIGO IB1b/IIA1 and tumour extension <2 cm can [9, 10]. The following recommendations are based on the currently valid German guidelines on cervical cancer and fertility maintenance in oncological disease [7, 11]. The Table 2 shows the flow chart of therapeutic decisions in young women with cervical cancer.

- In microinvasive stage FIGO IA1 with one risk factor, conisation—ideally as LLETZ (loop conisation) with R0 resection—primarily allows fertility to be maintained. For FIGO IA1 with L1 or FIGO IA2 and LO, a single sentinel node biopsy can be considered [12].
- In FIGO IA1 with two risk factors and from FIGO IA2 with one risk factor, the risk of lymph node involvement increases up to 5%. From these stages on, lymph node staging should be performed, ideally before the decision on fertility preservation. Radical trachelectomy according to Dargent [13] as a combination of tumour resection and complete staging as well as permanent cerclage is a special example of a surgical tumour treatment that is explicitly aimed at fertility. The post-operative residual cervix with a functional length of < or >10 mm is the most important influencing parameter on later obstetric complications. Vaginal sonography and MRI can be used to assess the risk of preterm birth [14].
- Under certain conditions—no other risk factors and small tumour volume (tumour <2 cm)—a radical trachelectomy can also be performed to treat FIGO IB1 and FIGO IIA1. Completion of surgery is recommended after pregnancy.

- Trachelectomy should not be recommended for neuroendocrine tumours or non-HPV-associated adenocarcinomas of the cervix.
- The uterine corpus should no longer be preserved for FIGO > IB1 cancers and radical hysterectomy, e.g. as total mesometrial resection (TMMR), is indicated [15]. The operation is performed according to the embryologically developed compartments and in a manner which protects the nerves. Even in the presence of risk factors, the TMMR treatment concept does not include adjuvant radiochemotherapy, only adjuvant chemotherapy. If radical hysterectomy is indicated, a bilateral salpingectomy should be performed, as the tubes belong to the uterovaginal (Müllerian) compartment.

Preservation of the ovaries is attempted, if possible, in cervical cancer patients. Even if there is evidence of adenocarcinoma, ovary preservation surgery can be performed in early stages after risk assessment. Various larger retrospective analyses of women with ovarian preservation and adenocarcinoma of the cervix FIGO stage I and II showed no significant differences in mortality [16]. Bilateral salpingo-oophorectomy should be considered from FIGO IB2 and adenocarcinoma onwards.

Combined Radiochemotherapy

If combined pelvic radiochemotherapy is necessary, the ovaries should be moved laterally and cranially outside the planned radiation field to protect the endocrine function. However, it is now also known that transposition of the ovaries itself can lead to a reduction in the ovarian reserve [17]. The gonadotoxic effect of pelvic radiotherapy depends on the total dose and the local dose calculated in the ovarian area, as well as the age of the woman during radiotherapy. At the age of 30, the radiation dose at which 97.5% of treated women experience complete ovarian failure (sterilisation) is 14.3 Gy [18] (see chapter “Indications for and Against Fertility Preservation”, Table 1). If the uterus is preserved and radiotherapy is performed, the uterus is no longer compatible with a later pregnancy. A uterine transplantation (see chapter “Further Fertility Preservation Techniques”) would then be an experimental approach, but it is particularly difficult in women who have previously undergone surgery and involves additional risks from the necessary immunosuppression [19].

Combined radiochemotherapy typically uses platinum-containing regimens for sensitisation to enhance the efficacy. Nevertheless, the gonadotoxic effect increases with the simultaneous irradiation of the pelvis.

Neoadjuvant Chemotherapy

Currently, neoadjuvant chemotherapy is only given in individual cases to enable uterus preservation after downstaging [20]. Ovarian tissue can be removed during laparoscopic lymph node staging [21] before the start of chemotherapy. However, this must be regarded as an experimental procedure.

In higher stages, active fertility preservation is not possible and endangers oncological safety.

Effectiveness and Risks of Fertility Preservation Therapy

Fertility-preservation therapy for cervical cancer is essentially limited to therapy which preserves the ovaries and uterine corpus. Although it is possible to carry out the fertility preservation measures listed in Table 2, these are often associated with considerable risks due to the increased probability of recurrence.

Metastasis of early cervical cancer into the ovaries is rare and is more likely to be found in the presence of risk factors such as deep infiltration and involvement of the uterine corpus. Nevertheless, adenocarcinoma in young women in particular is associated with an increased risk of ovarian metastasis [22]. This risk must be especially pointed out for adenocarcinoma with each ovarian preservation and cryopreservation. A meta-analysis of studies on cryopreserved ovarian tissue has not yet found an increased risk in women with cervical cancer, but the statement is limited due to the small number of cases and histological significance [23].

Other possible risks, which must be discussed individually with the patient as well as with the interdisciplinary teams (gynaecology oncology, radiotherapy and reproductive medicine), are shown in Table 2.

Conisation and trachelectomy can be performed as uterus-preserving and thus primarily fertility-preserving measures.

There have been several international studies in the past 15 years with higher case numbers which describe the oncological outcome and onset of pregnancy after trachelectomy. Recurrence rates after trachelectomy are reported to be <5% according to a Canadian publication [24]. However, the risk of recurrence after trachelectomy increases to 11% in women with primary tumours >2 cm. Pregnancies after trachelectomy are possible, and according to this analysis of more than 1200 treated

Table 2 Possible risks of fertility preservation for women with cervical cancer

Suggested oncological treatment	Options for fertility preservation	Possible risks
Radical hysterectomy	Preservation of uterus Cervical conisation Trachelectomy	Reduced oncological safety Increased risk of recurrence Obstetric risk after conisation and especially trachelectomy with increased risk of miscarriage and preterm birth
Bilateral salpingo-oophorectomy	Preservation of the ovary	Risk of ovarian metastasis (especially in adenocarcinoma)
Pelvic radiotherapy	Transposition of the ovaries	Alteration of ovarian function Chronic pain
Chemotherapy	Freezing of ovarian tissue	Transplantation of ovarian metastasis (especially in adenocarcinoma)

women, resulted in a live birth rate of 66.7%. A French study [25] also gives a comparable live birth rate after trachelectomy (70%) with a high proportion of premature births (38%).

Practical Approach

For women with a desire to have children and newly diagnosed early cervical carcinoma FIGO IA1 with risk factors, or up to IA2 without risk factors, fertility-preserving surgery with conisation is possible. Radical trachelectomy and ovarian preservation can be performed up to FIGO IB1 and FIGO IIA1 < 2 cm without risk factors. An increased risk of recurrence must be assessed in each individual case. The ovaries can be moved laterally prior to planned radiotherapy to maintain endocrine function.

If the ovarian reserve can be preserved in cervical cancer (possibly also by cryopreservation of ovarian tissue) but not the uterus, or if radiotherapy is required, there is theoretically—although not in Germany—the possibility of surrogacy. However, because of its ethical implications against legal regulations in many countries of the world, surrogate motherhood must be discussed in detail with the patient or the couple.

Experimental transplantation of the uterus (see chapter "Further Fertility Preservation Techniques") has also been reported [26, 27]. Also controversially discussed and classified as experimental is neoadjuvant chemotherapy for cervical cancer in higher stages, which should enable fertility-preserving surgery after downstaging (Fig. 2).

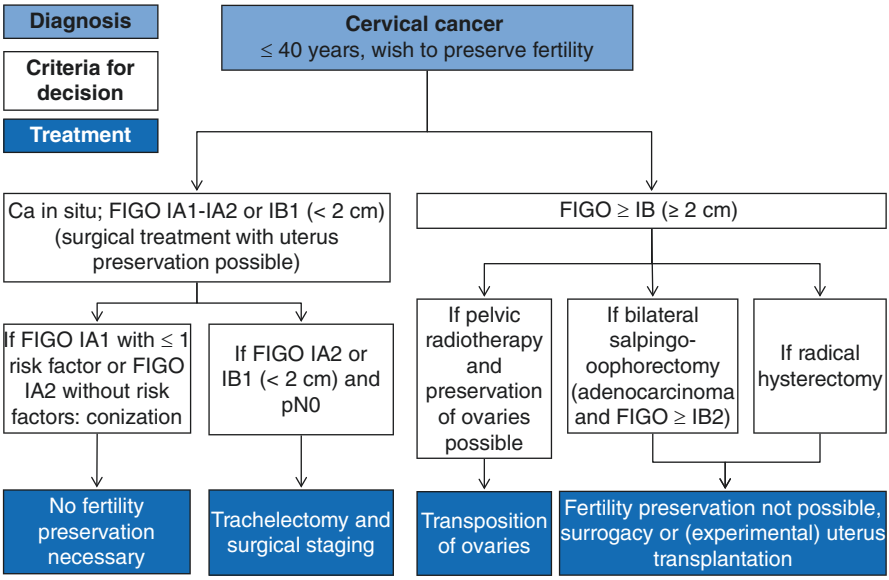


Fig. 2 Flowchart for fertility-preservation procedures in cervical carcinoma

References

1. von Woff M, Dittrich R, Liebenthron J, Nawroth F, Schüring AN, et al. Fertility preservation counselling and treatment for medical reasons: data from a multinational network of over 5000 women. Reprod Biomed Online. 2015;31:605–12. https://doi.org/10.1016/j.rbmo.2015.07.013.
2. Rob L, Skapa P, Robova H. Fertility-sparing surgery in patients with cervical cancer. Lancet Oncol. 2011;12:192–200. https://doi.org/10.1016/S1470-2045(10)70084-X.
3. Kyrgiou M, Shafi MI. Invasive cancer of the cervix. Obstet Gynaecol Reprod. 2010;20:147–57. https://doi.org/10.1016/j.ogrm.2010.02.003.
4. Arbyn M, Xu L, Simoens C, Martin-Hirsch PP. Prophylactic vaccination against HPV to prevent cervical cancer and its precursors. Cochrane Database Syst Rev. 2018;5:CD009069. https://doi.org/10.1002/14651858.CD009069.pub3.
5. Waggoner SE. Cervical cancer. Lancet. 2003;361:2217–25. https://doi.org/10.1016/S0140-6736(03)13778-6.
6. http://www.krebsdaten.de/Krebs/DE/Content/Krebsarten/Gebaermutterhalskrebs/gebaermutterhalskrebs_node.html, Last accessed 25.1.2020.
7. S3-Leitlinie. Diagnostik, Therapie und Nachsorge der Patientin mit Zervixkarzinom Version 1.0—September 2014, AWMF-Registernummer 032/033OL.
8. Kyrgiou M, Athanasiou A, Paraskevaidi M, Mitra A, Kalliala I, et al. Adverse obstetric outcomes after local treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta-analysis. BMJ. 2016;354:i3633. https://doi.org/10.1001/jamaoncol.2016.1839.
9. Bhatla N, Berek JS, Cuello Fredes M, Denny LA, Grenman S, Karunaratne K, et al. Revised FIGO staging for carcinoma of the cervix uteri. Int J Gynaecol Obstet. 2019 Apr;145(1):129–35. https://doi.org/10.1002/ijgo.12749.
10. S2k Leitlinie: Fertilitätserhaltung bei onkologischen Therapien. Level S2k, AWMF Register Nr. 015/082, November 2017. http://www.awmf.org/leitlinien/detail/ll/015-082.html. Last accessed 25.1.2020.
11. Bhatla N, Berek JS, Cuello Fredes M, Denny LA, Grenman S, Karunaratne K, et al. Revised FIGO staging for the carcinoma of the cervix uteri. Int J Gynae. 2019;145(1):129–35. https://doi.org/10.1002/ijgo.12749.
12. Cibula D, Pötter R, Planchamp F, Avall-Lundqvist E, Fischerova D, et al. The European society of gynaecological oncology guidelines for the management of patients with cervical cancer. Radiother Oncol. 2018 Jun;127(3):404–16. https://doi.org/10.1016/j.radonc.2018.03.003.
13. Dargent D, Martin X, Sacchetoni A, Mathevet P. Laparoscopic vaginal radical trachelectomy: a treatment to preserve the fertility of cervical carcinoma patients. Cancer. 2000;88:1877–82. https://doi.org/10.1016/s1040-8428(03)00129-x.
14. Alvarez RM, Biliatis I, Rockall A, Papadakou E, Sohaib SA, deSouza NM, et al. MRI measurement of residual cervical length after radical trachelectomy for cervical cancer and the risk of adverse pregnancy outcomes: a blinded imaging analysis. BJOG. 2018;125:1726–33. https://doi.org/10.1111/1471-0528.15429.
15. Höckel M, Horn LC, Manthey N, Braumann UD, Wolf U, et al. Resection of the embryologically defined uterovaginal (Müllerian) compartment and pelvic control in patients with cervical cancer: a prospective analysis. Lancet Oncol. 2009;10:683–92. https://doi.org/10.1016/S1470-2045(09)70100-7.
16. Chen J, Wang R, Zahng B, Lin X, Wei J, Jia Y, et al. Safety of ovarian preservation in women with stage I and II cervical adenocarcinoma: a retrospective study and meta-analysis. Am J Obstet Gynceol. 2016;215:460.e1–e13. https://doi.org/10.1016/j.ajog.2016.04.023.
17. Buekers TE, Anderson B, Sorosky JI, Buller RE, Pahisa J, Martinez-Roman S, et al. Ovarian function after surgical treatment for cervical cancer. Gynecol Oncol. 2001;80:85–8. https://doi.org/10.1006/gyno.2000.6039.

18. Wallace WH, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. Int J Radiat Oncol Biol Phys. 2005;62:738–44. https://doi.org/10.1016/j.ijrobp.2004.11.038.
19. Castellon LAR, Amador MIG, Gonzales RED, Eduardo MSJ, Díaz-García C, Kvarnström N, et al. The history behind successful uterine transplantation in humans. JBRA Assist Reprod. 2017;21:126–34. https://doi.org/10.5935/1518-0557.20170028.
20. Laios A, Kasius J, Tranoulis A, Gryparis A, Ind T. Obstetric outcomes in women with early bulky cervical cancer downstaged by neoadjuvant chemotherapy to allow for fertility-sparing surgery: a meta-analysis and metaregression. Int J Gynaecol Cancer. 2018;28:794–801. https://doi.org/10.1097/IGC.0000000000001232.
21. Vercellino GF, Piek JM, Schneider A, Köhler C, Mangler M, Speiser D, et al. Laparoscopic lymph node dissection should be performed before fertility preserving treatment of patients with cervical cancer. Gynecol Oncol. 2012;126:325–9. https://doi.org/10.1016/j.ygyno.2012.05.033.
22. Ronnett BM, Yemelyanova AV, Vang R, Gilks CB, Miller D, Gravitt PE, et al. Endocervical adenocarcinomas with ovarian metastases: analysis of 29 cases with emphasis on minimally invasive cervical tumors and the ability of the metastases to simulate primary ovarian neoplasms. Am J Surg Pathol. 2008;32:1835–53. https://doi.org/10.1097/PAS.0b013e3181758831.
23. Bastings L, Beerendonk CC, Westphal JR, Massuger LF, Kaal SE, van Leeuwen FE, et al. Autotransplantation of cryopreserved ovarian tissue in cancer survivors and the risk of reintroducing malignancy: a systematic review. Hum Reprod Update. 2013;19:483–506. https://doi.org/10.1093/humupd/dmt020.
24. Willows K, Lennox G, Covens A. Fertility-sparing management in cervical cancer: balancing oncologic outcomes with reproductive success. Gynecol Oncol Res Pract. 2016;3:9. https://doi.org/10.1186/s40661-016-0030-9.
25. Bentivegna E, Maulard A, Pautier P, Chargari C, Gouv S, Morice P. Fertility results and pregnancy outcomes after conservative treatment of cervical cancer: a systematic review of cervical cancer: a systematic review of the literature. Fertil Steril. 2016;106:1195–1211.e5. https://doi.org/10.1016/j.fertnstert.2016.06.032.
26. Brännström M, Enskog A, Kvarnström N, Ayoubi JM, Dahm-Kähler P. Global results of human uterus transplantation and strategies for pre-transplantation screening of donors. Fertil Steril. 2019;112:3–10. https://doi.org/10.1016/j.fertnstert.2019.05.030.
27. Brännström M, Dahm-Kähler P. Uterus transplantation and fertility preservation. Best Pract Res Clin Obstet Gynaecol. 2019;55:109–16. https://doi.org/10.1016/j.fertnstert.2019.05.029.

Endometrial Hyperplasia and Endometrial Carcinoma

Maren Goeckenjan, Michael von Wolff, and Pauline Wimberger

Stage-Dependent Prognosis

The tendency of postponed family planning in developed countries in combination with overnutrition, overweight and the earlier occurence of metabolic diseases in life, all these factors lead to an increase in complex endometrial hyperplasia with atypia and endometrial cancer, even in women of reproductive age. Untreated, endometrial hyperplasia without atypia results in endometrial carcinoma in about 1% of cases and with complex atypia in about 30% [1].

Type I carcinoma is estrogen-dependent and has the typical risk factors that are also frequently associated with unfulfilled desire to have children, such as nulliparity, polycystic ovarian syndrome, diabetes mellitus, arterial hypertension and obesity.

Although endometrial carcinoma is rarely diagnosed premenopausally, women under the age of 45 years account for about 6% of all patients diagnosed with endometrial carcinoma [2].

Young women typically have endometrioid endometrial carcinoma with a low tumour stage and grade and therefore a good prognosis. If the endometrioid carcinoma occurs in young women, a genetic predisposition for tumour development of the hereditary tumour syndrome HNPCC (hereditary non-polyposis colorectal carcinoma or Lynch syndrome) should be offered. The estimated lifetime risk for endometrial carcinoma in women with a genetic mutation for Lynch syndrome is 40–60% (Table 1) [1].

M. Goeckenjan (✉) · P. Wimberger
TU Dresden, University Hospital, Dresden, Germany
e-mail: maren.goeckenjan@uniklinikum-dresden.de; pauline.wimberger@uniklinikum-dresden.de

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_11

Table 1 Endometrial hyperplasia and endometrial carcinoma: prognosis and oncological treatment for women with and without fertility preservation [1, 3]

	Prognosis	Oncological treatment according to guidelines	Fertility preservation
Hyperplasia of endometrium without atypia	Risk of progression to endometrial cancer: 1%	Hysterectomy not necessary. Cyclic progestin treatment, i.e. 10–20 mg MPA/day. Endometrial biopsies every 3–6 months with hysteroscopy and curettage until pregnancy	No progestin treatment. Time to pregnancy should be as short as possible.
Atypical endometrial hyperplasia (AEH)	Risk of progression to endometrial carcinoma in complex AEH: 30%. Histology reveals invasive carcinoma in 20–40%	100 mg MPA/day or levonorgestrel-releasing intrauterine devices, IUD. Endometrial biopsies every 3–6 months with hysteroscopy and curettage until pregnancy.	Conception after progestin treatment, curettage without signs of hyperplasia. Time to pregnancy should be reduced. Periodic endometrial biopsies. Completion of surgical treatment after delivery.
Endometrial carcinoma	Increased risk of recurrence if uterus sparing surgery is chosen: 15% in all stages. 5-year survival rate in all stages 75–83%.	Standard treatment for pT1a, G1 or G2 (low-risk endometrial carcinoma): Hysterectomy and bilateral salpingo-oophorectomy. In higher stages: Plus pelvic and paraaortic lymphonodectomy. In low-risk carcinoma: Adjuvant treatment not necessary.	If pT1a, G1 and progesterone sensitive: Progestin treatment, e.g. 250 mg MPA/day or levonorgestrel-releasing intrauterine device (IUD). Hysteroscopy and curettage every 3 months for 1 year or until pregnancy. Completion of surgical treatment after delivery.

Gonadotoxicity of Treatment

Gonadotoxic chemotherapy is not necessary at an early stage. Either progestins are used for drug treatment, which is not gonadotoxic, or a hysterectomy with bilateral salpingo-oophorectomy is performed. For endometrial carcinoma in stage FIGO IA, G1/2, systematic lymphadenectomy is not indicated if the lymph nodes are clinically not affected [1]. Adjuvant chemotherapy is also recommended if there is pelvic and/or paraaortic lymph node involvement. The detection of lymph node metastases—especially macroscopically suspect lymph nodes—is particularly unfavourable for the prognosis of endometrial carcinoma and is staged as FIGO IIIc. In these women, treatment consists of a macroscopic complete resection including hysterectomy, bilateral salpingo-oophorectomy and systematic lymphonodectomy, as well as the indication for adjuvant chemotherapy with carboplatin and paclitaxel. Adjuvant radiotherapy is also recommended to reduce the risk of

local recurrence. In advanced stages, a fertility-preserving procedure is therefore not feasible.

Treatments and Effects on Fertility

The standard treatment in endometrial hyperplasia with atypia is progestin treatment with hysteroscopic and histological follow-ups. Treatment for endometrial carcinoma basically includes hysterectomy and salpingo-oophorectomy.

In early endometrial carcinoma FIGO IA, G1/G2, the prognosis after hysterectomy and bilateral salpingo-oophorectomy is excellent, with a 5-year survival rate of 99% [1]. In higher stages, systematic pelvic and paraaortic lymphadenectomy are indicated.

Data on uterus preservation and the risks in women with endometrial carcinoma who have an urgent desire to have children are scarce. In principle, this approach is only justifiable in well-differentiated endometrioid endometrial carcinoma, FIGO IA, G1 with progesterone receptor detection and in stages limited to the endometrium without infiltration of the myometrium. Accompanying hormone therapy with progestins, mostly high-dose medroxyprogesterone acetate 250 mg/day for at least 6–9 months, is recommended [4]. Progestin treatment is associated with high remission rates in early endometrial carcinoma; no further remissions are to be expected after 9 months of progestin treatment [1].

In recent years, several mostly retrospective studies with small case numbers have been published on fertility-preservation approaches with endocrine treatment for endometrial carcinoma FIGO IA, G1 and endometrial hyperplasia with atypia, which is considered a precancerous condition for endometrial carcinoma. A recent meta-analysis with 54 observational studies showed high birth rates of over 50% and low recurrence rates with progestin treatment and regular hysteroscopies with curettage [5]. An overview of the studies on fertility preservation in endometrial hyperplasia with atypia and endometrial carcinoma over the last 5 years is shown in Table 2.

Due to the lack of studies with large case numbers on a standard approach, fertility preservation in women with endometrial cancer who wish to have children is still an individual case decision. The following procedures are well-established if fertility should be preserved [1, 3]:

- Fertility preservation can be considered in well-differentiated endometrioid endometrial carcinoma (pT1a, G1) with progesterone receptor expression.
- Adnexal involvement or myometrial infiltration needs to be excluded.
- Patient needs to be informed that postponing standard surgical treatment with curative hysterectomy is associated with risks (progression of the disease, metastasis).
- Patient needs to be informed about the need for close monitoring and hysterectomy after family planning is completed.

Table 2 Fertility preservation in women with endometrium carcinoma (EC) or atypical endometrium hyperplasia (AEH)

	Study design	Treatment	Included women	Comments
Gonthier et al. 2015 [6]	Retrospective multi-Centre study (France).	Treatment with progestins.	$n = 32$ EC G1, $n = 111$ AEH, Age ≤ 40 years comparison of women with fertility preservation ($n = 32$) and those with hysterectomy (controls).	Risk for recurrence of carcinoma after fertility preservation in women with EC is increased compared to controls. Risk of recurrence after fertility preservation for AEH is not statistically significant.
De Marzi et al. 2015 [7]	Retrospective observational study, no controls (Italy).	Hysteroscopic resection and progestin treatment with 160 mg MPA/day.	$n = 3$ with EC G1, $n = 20$ with AEH, Age ≤ 45 years.	52% complete remission after 3 months, 39% after 6 months, Median time of observation: 25 months, 7 pregnancies in 6 women.
Chen et al. 2015 [8]	Retrospective study, no controls (China).	Treatment with progestins for at least 6 months.	$n = 37$ EC FIGO IA G1, $n = 16$ AEH, Age ≤ 41 years.	75% complete remission after 6 months, 5 years without recurrence: 71%, 17 pregnancies in 33 women.
Pronin et al. 2015 [9]	Prospective study, no controls (Russia).	Treatment with levonorgestrel-IUD and GnRH agonists for at least 6 months.	$n = 32$ EC G1, $n = 38$ AEH, Age ≤ 42 years.	72% complete remission in EC and 92% in AEH, 8 women pregnant.
Zhou et al. 2015 [10]	Retrospective study, no controls (China).	Treatment with progestins, combined treatment with metformin for at least 12 weeks if HbA1c increased.	$n = 19$ EC G1, $n = 13$ AEH, Age ≤ 40 years.	84% complete remission, Median observation of 32.5 months. 9 of 21 women pregnant, prognosis optimised with concomitant use of metformin.

Table 2 (continued)

	Study design	Treatment	Included women	Comments
Yang et al. 2019 [11]	Retrospective study with AH and EC. No controls (China).	Progestin treatment and hysteroscopy and curettage every 3 months.	*n* = 40 EC FIGO IA, *n* = 120 AEH, Median age 32 years.	Median duration of progestin treatment 6.7 months until complete remission. 45% pregnancy and 25% live birth rate.

MEDLINE-literature search: prospective or retrospective studies with at least 20 women, 2015–2019

- Adequate drug treatment with progestins (medroxyprogesterone acetate or megestrol acetate) or a levonorgestrel-releasing intrauterine system is required.
- Follow-up every 3 months with hysteroscopy and endometrial biopsy is required.
- The shortest time to pregnancy after discontinuation of progestin therapy is required. Cooperation with a reproductive physician might be necessary.
- Due to the high risk of relapse after family planning is complete, stage-adjusted treatment (hysterectomy and bilateral salpingo-oophorectomy) is required.

There is an increased oncological risk from inadequate staging if hysterectomy and salpingo-oophorectomy are not performed. The chance of complete remission is 50–84%, depending on the study [1]. However, the presence of the ovaries in low stages of endometrioid endometrial carcinoma does not worsen the survival and recurrence-free interval [12]. Nevertheless, the risk of later ovarian cancer or metastasis into the ovary must be considered if the ovaries remain in young women. Attention is drawn to the risk of synchronous ovarian cancer with endometrial cancer in young women with a genetic predisposition (HNPCC) [13].

Practical Approach

Although hysterectomy is part of the standard treatment for early endometrial carcinoma, temporary uterus preservation can be achieved in young women who wish to have a child with FIGO IA G1, early well-differentiated endometrial carcinoma with expression of progesterone receptors without myometrial involvement. Progestin treatment with medroxyprogesterone acetate (MPA) or megestrol acetate is usually started. Despite progestin treatment, the recurrence rate without increasing mortality is so high that a hysterectomy should be performed after pregnancy and birth. Close follow-up every 3 months with hysteroscopy and endometrial biopsy during endocrine treatment is indicated. Medication can only be discontinued after several months (preferably 12 months) of progestin treatment when the oncological situation is stable and the woman gets pregnant soon (Fig. 1).

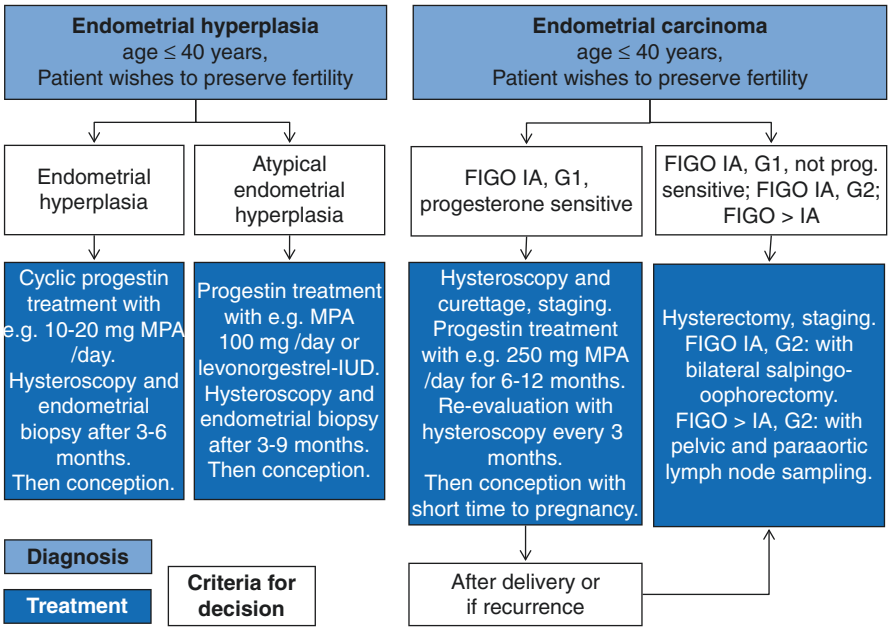


Fig. 1 Algorithm for fertility preservation in cervical carcinoma

References

1. GGPO German Guideline program in oncology. Guideline on the diagnosis, treatment, and follow-up of patients with endometrial carcinoma, short version 1.0 2018, AWMF-register no. 032/034-OL.
2. Gerstl B, Sullivan E, Vallejo M, Koch J, Johnson M, Wand H, et al. Reproductive outcome following treatment for a gynecological cancer diagnosis: a systematic review. J Cancer Surviv. 2019;13:269–81. https://doi.org/10.1007/s11764-019-00749-x.
3. S2k-Leitlinie. Fertilitätserhalt bei onkologischen Erkrankungen, Langversion V 1.0 2017, AWMF-Registernummer 015/082.
4. Simpson AN, Feigenberg T, Clarke BA, Gien LT, Ismiil N, Laframboise S, et al. Fertility sparing treatment of complex atypical hyperplasia and low grade endometrial cancer using oral progestin. Gynecol Oncol. 2014;133:229–33. https://doi.org/10.1016/j.ygyno.2014.02.020.
5. Zhang Q, Qi G, Kanis MJ, Dong R, Cui B, Yang X, et al. Comparison among fertility-sparing therapies for well differentiated early-stage endometrial carcinoma and complex atypical hyperplasie. Oncotarget. 2017;8:57642–53. https://doi.org/10.18632/oncotarget.17588.
6. Gonthier C, Piel B, Touboul C, Walker F, Cortez A, Luton D, et al. Cancer incidence in patients with atypical endometrial hyperplasia managed by primary hysterectomy of fertility-sparing treatment. Anticancer Res. 2015;35:6799–804.
7. De Marzi P, Bergamini A, Luchini S, Petrone M, Taccagni GL, Mangili G, et al. Hysteroscopic resection in fertility-sparing surgery for atypical hyperplasia and endometrial cancer: safety and efficacy. J Minim Invasive Gynecol. 2015;22:1178–82. https://doi.org/10.1016/j.jmig.2015.06.004.

8. Chen M, Jin Y, Li Y, Bi Y, Shan Y, Pan L. Oncologic and reproductive outcomes after fertility-sparing management with oral progestin for women with complex endometrial hyperplasia and endometrial cancer. Int J Gynaecol Obstet. 2016;132:34–8. https://doi.org/10.1016/j.ijgo.2015.06.046.
9. Pronin SM, Novikova OV, Andreeva JY, Novikova EG. Fertility-sparing treatment of early endometrial cancer and complex atypical hyperplasia in young women of childbearing potential. Int J Gynecol Cancer. 2015;25:1010–4. https://doi.org/10.1097/IGC.0000000000000467.
10. Zhou R, Yang Y, Lu Q, Wang J, Miao Y, Wang S, et al. Prognostic factors of oncological and reproductive outcomes in fertility-sparing treatment of complex atypical hyperplasia and low-grade endometrial cancer using oral progestin in Chinese patients. Gynecol Oncol. 2015;139:424–8. https://doi.org/10.1016/j.ygyno.2015.09.078.
11. Yang B, Xu Y, Zhu Q, Xie L, Shan W, Ning C, et al. Treatment efficiency of comprehensive hysteroscopic evaluation and lesion resection combined with progestin therapy in young women with endometrial atypical hyperplasia and endometrial cancer. Gynecol Oncol. 2019;153:55–62. https://doi.org/10.1016/j.ygyno.2019.01.014.
12. Gu H, Li J, Gu Y, Tu H, Zhou Y, Liu J. Survival impact of ovarian preservation on women with early-stage endometrial cancer: a systematic review and meta-analysis. Int J Gynecol Cancer. 2017;27:77–84. https://doi.org/10.1097/IGC.0000000000000857.
13. Dogan A, Schultheis B, Rezniczek GA, Hilal Z, Cetin C, Häusler G, et al. Synchronous endometrial and ovarian cancer in young women: case report and review of the literature. Anticancer Res. 2017;37:969–78.

Paediatric Cancer

Magdalena Balcerek, Michael von Wolff, and Anja Borgmann-Staudt

Stage-Dependent Prognosis

In paediatric oncology in Germany, acute leukaemia are the most common malignant disease with 30.3%, followed by brain tumours with 23.8% and lymphomas with 14.3%. Less frequent are soft tissue sarcomas (5.8%), peripheral nerve cell tumours (5.6%), bone (5.2%), kidney (4.7%) and germ cell tumours (4.0%) [1]. With the implementation of therapy optimisation studies (TOS) by the German Society for Paediatric Oncology and Haematology (GPOH) since the 1970s, the survival rates for girls and boys with malignant diseases have steadily improved. The 15-year survival rate is currently 82% of those affected with a paediatric oncological disease that was diagnosed before the age of 15 [1]. Approximately 35,000 former paediatric oncology patients are currently undergoing long-term follow-up in the German Childhood Cancer Registry [1] (Table 1).

In the *Ferti*PROTEKT network, children and adolescents under the age of 14 made up 2.5% of patients that underwent counselling in 2016–2018. About a third of these were due to Hodgkin's lymphoma and about a quarter due to leukaemia.

M. Balcerek (✉) · A. Borgmann-Staudt
Charité – Universitätsmedizin Berlin, Freie Universität Berlin,
Humboldt-Universität zu Berlin, Berlin, Germany

Berlin Institute of Health (BIH), Berlin, Germany

Department of Paediatric Oncology, Haematology and Stem Cell Transplantation,
Augustenburger Platz 1, Berlin, Germany

Division of Gynaecological Endocrinology and Reproductive Medicine,
University Women's Hospital, University of Bern, Bern, Germany
e-mail: magdalena.balcerek@charite.de; anja.borgmann@charite.de

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_12

Table 1 Long-term survival rates and therapy protocols for the different paediatric cancers, including tumour stages

Diagnosis	International Classification of Childhood Cancer (ICCC-3)	Proportion 2004–2013 in Germany (%)	Survival rate		Therapy protocols of the German Society for Paediatric Oncology and Haematology (*GPOH*)
			5 years (%)	15 years (%)	
Paediatric cancers in general	–	100	85	82	
Leukaemia, myeloproliferative diseases and myelodysplastic diseases	I	30.3	89	86	
Lymphoid leukaemia	I(a)		92	90	AIEOP-BFM ALL, ALL-BFM, Co-ALL
Acute myeloid leukaemia	I(b)		73	71	AML-BFM
Chronic myeloproliferative diseases	I(c)		96	96	CML-paed II
Myelodysplastic syndrome and other myeloproliferative diseases	I(d)		82	76	EWOG-MDS 2006
Lymphomas and reticuloendothelial neoplasms	II	14.3	94	92	
Hodgkin's lymphoma	II(a)		99	97	EuroNET-PHL-C1, HD
Non-Hodgkin's lymphomas (except Burkitt lymphoma)	II(b)		89	96	NHL-BFM, B-NHL BFM, NHL-BFM
Burkitt lymphoma	II(c)		93	92	NHL-BFM, B-NHL BFM, NHL-BFM
CNS tumours	III	23.8	77	71	
Ependymomas and choroid plexus tumour	III(a)		81	69	HIT, HIT-MED; HIT-SKK
Astrocytoma	III(b)		81	77	SIOP LGG,
Intracranial and intraspinal embryonal tumours	III(c)		67	56	HIT, HIT-MED; HIT-SKK
Other gliomas	III(d)		46	43	HIT-GBM, HIT-HGG
Other specified intracranial and intraspinal neoplasms (e.g. adenoma of the pituitary gland; craniopharyngioma)	III(e)		96	91	Craniopharyngioma, HIT-Endo

Table 1 (continued)

Diagnosis	International Classification of Childhood Cancer (ICCC-3)	Proportion 2004–2013 in Germany (%)	Survival rate		Therapy protocols of the German Society for Paediatric Oncology and Haematology (*GPOH*)
			5 years (%)	15 years (%)	
Neuroblastoma and other peripheral nervous cell tumours	IV	5.6	80	76	NB
Malignant bone tumours	VIII	5.2	72	67	Ewing-sarcoma: Ewing, Euro EWING, EICESS, CESS Osteosarcoma: EURAMOS-1, COSS
Soft tissue and other extraosseous sarcomas	IX	5.8	73	69	CWS-SoTiSaR, CWS
Germ cell tumours	X	4.0	94	93	MAHO, MAKEI
Others (retinoblastoma, renal tumours, hepatoblastoma, carcinoma)	V, VI, VII, XI	–	–	–	Retinoblastoma Registry Renal tumours: SIOP 2001/GPOH Hepatoblastoma: HB

The reason for further consultations was medulloblastoma, myelodysplastic syndromes, sarcoma, neuroblastoma and astrocytoma [2].

Gonadotoxicity of Therapies

In up to one-third of girls and boys, fertility is impaired after chemo- and/or radiotherapy [3–5], and in more than two-thirds after stem cell transplantation [6]. In individual cases, recovery of gonadal function is possible within a few years after chemo- and radiotherapy [7]. Almost all former patients wish to have their own child [8]. While the miscarriage rate may be somewhat higher in former patients [8], the rate is significantly lower than in the general population in Germany [9].

The risk factors for a relevant functional impairment of the gonads are described below. For faster orientation, the respective therapy arms of the therapy protocols used in paediatric oncology (TOS protocols) are also listed according to the risk of a relevant functional impairment of the gonads.

If there is a high risk, fertility preservation measures are strongly recommended before starting gonadotoxic therapy. In principle, however, it is not always possible to identify the risk group to which the patient belongs before therapy. Therefore, fertility-preservation measures should also be considered in children and adolescents with an initial medium risk and, if necessary, can also be discussed with the families of patients with a lower risk. The risks of fertility-preservation measures, the feasibility in connection with oncological therapy, and the wishes of those affected must always be taken into account.

The following tables describe therapies with a high (red), a medium (yellow) and a low risk (green) for gonadotoxicity.

Table 2 Therapies associated with a *high risk* of gonadotoxicity

Irradiation to the pelvis and total body irradiation [6, 10–12] (also see chapter "Logistics for Fertility Preservation Counselling"):
• Irradiation dose *to the ovaries* ≥10 Gy (age-dependent risk, with a postpubertal organ being more sensitive to irradiation [10])
• Irradiation below L5: Increased risk; iliac irradiation: High risk; inguinal irradiation: Individually differing level of risk
• *Testicular* irradiation dose ≥4 Gy
Chemotherapeutic agents:
• In *girls*: *Busulfan ≥14 mg/kg/KG cumulative dose* [6, 7]
• In *boys*: *Procarbazine ≥6 g/m2* [13]

Table 3 Chemotherapeutic agents and doses associated with a *medium risk* of gonadotoxicity [11, 14, 15]

Busulfan >0.4 g/m^2 [6, 7]
Carboplatin >2 g/m^2 (data are uncertain)
Cisplatin >0.5 g/m^2 [10]
Cyclophosphamide >10 g/m^2 [15]
Etoposide >5 g/m^2 [10]
Ifosfamide >42 g/m^2 [4]
Melphalan >0.14–0.24 g/m^2
Procarbazine in *girls* >6 g/m^2 [13]
Procarbazine in *boys*: >3 g/m^2 [13]

Classification According to Therapy and Study Protocols

Patients who have undergone haematopoietic stem cell transplantation where busulfan or total body irradiation (TBI) was used for conditioning therapy have a *high risk* of fertility problems later in life. Local pelvic irradiation is also highly gonadotoxic [6, 7, 10–12] (Table 2):

The following chemotherapeutic agents present a *medium risk* of a fertility disorder later in life [6, 7, 10, 11, 13–15] (Table 3):

The chemotherapeutic agents listed in Table 3 are used in the appropriate doses in the following TOS protocols and study arms (for associated diagnoses, see Tables 1 and 4):

Patients who receive therapy according to the TOS protocols shown in Table 5 are at *low risk*:

Classification According to Patient Factors and Treatments

Treatment at prepubertal age is associated with a lower risk of later fertility impairment in girls and boys [10]. In cases of prepubertal radiotherapy to the pituitary–hypothalamic axis in girls, a deviation in the age at menarche in the sense of both precocious puberty (for treatment see [19]) and delayed puberty is possible (for

Table 4 Therapy optimization trials (TOS)—protocols and study arms that include chemotherapeutic agents in doses associated with a *medium risk* of gonadotoxicity

CWS-SoTiSaR: RMS subgroup C1, D-H; other "RMS-like", "non-RMS-like" in HR, metastatic STS; *CWS 02*: SR B, HR; *96*: SR, HR; *91*: SR, HR HR; *86; 81*
EURAMOS-1: MAPIE; *COSS 96*: HR; *91*: IOR; *86*: LRV-VI, HR
Ewing 2008; Euro EWING 99; EICESS 92; CESS 86; 81
HB 1999: HB III SD/PD, IV PR; HCC: III/IV PR
EuroNET-PHL-C1: TG2 + 3 random 07-11; *HD 2002 Pilot TG3*, *HD 95*: TG3; *90*: TG3; *82*: TG3
HIT 2000: HIT2000-AB4, HIT2000-BIS4 -RT; MET-HIT2000-BIS4 CR/PR, P-HIT2000-AB4, P-HIT2000-BIS4-RT; E-HIT2000-AB4, E-HIT2000-BIS4 -RT
NB 2004: MR <6 M, HR; *97*: HR + Mega, HR + DT <6 M; *90*: RG2 + 3 A/B-CR, RG3 C-D + 4; *82*: III + LK, IV
SIOP LGG 2004: standard/intensified induction therapy; *96*
SIOP 2001/GPOH: II-IV + HR; *93-01*: I-V + HR, IV Non-CR

Table 5 Therapy optimization trials (TOS)—protocols and study arms that include chemotherapeutic agents in doses associated with a *low risk* of gonadotoxicity

AIEOP-BFM ALL 2009, ALL-BFM 2000, 95, 90, 86, 83, 81, 79, 77
AML-BFM 2004, 02, 98, 93, 87, 83, 78
Co-ALL-08-09, 03, 97, 92, 89, 85, 82, 80
CWS–SoTiSaR 2009: RMS subgroup A, B, C2; *02*: LR, SR A; *96*: LR; *91*: LR, HR LR
EURAMOS-1: MAP, MAPifn; *COSS 96*: LR, S1, S2; *91*: COSS, COSS/IOR; *90; 89; 86* LR I–IV; *85; 82; 80; 77*
EuroNET-PHL-C1 2007–2011 TG1, TG2 + 3 random, since 2012 TG1-3; *EuroNETPHL-LP1; HD 2002 Pilot; HD 95*: TG1; 90: TG1; 87; 85
HB 99: I + II; III PR; HCC: I/II; III/IV PR operable; SD/PD; PR (operable, SD/PD); *94; 89*
HIT-GBM D, C, B, A
HIT-HGG 2007
HIT 2000: HIT2000-BIS4 + RT; MET-HIT2000-BIS4 SD/PD, MET-HIT2000-AB4; PHIT2000-BIS4 + RT; E-HIT2000- BIS4 + RT; *HIT-MED 99; HIT-SKK 92; HIT 91; 89; 88; HIT-SKK 87*
Craniopharyngioma 2007, 2000; HIT-Endo 99, 96
NB 2004: Observation, MR N 6M; *97*: SR, HR + DT N 6M; *90*: RG2 + 3 A/B + CR, RGS-C 85; 82: II–II, III-LK; *79*
NHL-BFM Registry 2012, B-NHL BFM 04, NHL-BFM 95, 90, 86, 83, 81, 79, 77, 76, 75
MAHO 98; 94; 92; 88; 82
MAKEI 96; 89; 86; 83
SIOP 2001/GPOH: I, II–IV excluding HR; *93-01* I–V excluding HR; *89; 82; 80; 79*

treatment see [19]), although the majority of the former patients examined have a normal age at menarche after chemo- and/or radiotherapy [20].

A deficiency in GnRH or of FSH and/or LH can occur in patients after radiotherapy to the hypothalamus–pituitary axis with a pituitary dose ≥30 Gy, leading to a functional disorder of the gonads in the sense of a hypogonadotropic hypogonadism [3, 21]. This can be treated with hormone replacement therapy, even after a prolonged period (for treatment see [22]). Androgen deficiency has also been

Table 6 Relevant risk factors for hypogonadism following cancer treatment in childhood (modified according to [16])

Cancer-induced dysfunctions	Patient-related factors for increased risk for dysfunctions	Therapy-related factors in which fertility protection is recommended
Ovaries: Sterility, endocrine dysfunction	Age >12 years, postpubertal [4]	Bilateral tumours of the ovaries Therapy with alkylating agents [10, 11] Irradiation involving the ovaries [17] (also see chapter "Indications for and Against Fertility Preservation"): ≥10–15 Gy (prepubertal), ≥5–10 Gy (postpubertal)
Testicles: Endocrine dysfunction	Young age, prepubertal	Testicular carcinoma with unilateral orchiectomy Therapy with alkylating agents Irradiation to the testicles: Relevant dysfunctions: >20 Gy (prepubertal) and 30 Gy (postpubertal)
Testicles: Sterility	Postpubertal [4]	Testicular carcinoma with orchiectomy Therapy with alkylating agents [10, 13] Irradiation to the testicles (also see chapter "Indications for and Against Fertility Preservation"): ≥2 Gy; potentially reversible: <1.5 Gy; irreversible ≥4 Gy [18]

described after high-dose administration of procarbazine and after testicular irradiation with ≥20 Gy [23].

Damage to the uterus can occur after abdominal radiotherapy with more than 14 Gy, leading to an increased probability of pregnancy complications such as miscarriage, premature birth, low birth weight and increased perinatal mortality [3, 14, 24] (see also chapter "Pregnancy After Chemotherapy and Radiation of the Pelvis") (Table 6).

Probability of Gonadal Metastasis

The risk of gonadal metastasis in children and adolescents must always be given special consideration. For example, the risk of malignant cells in the gonads is high in systemic diseases such as leukaemia and blastic- and Burkitt's lymphoma [24, 25]. In 99 post-mortems of children with leukaemia, Kamiyama et al. [24] found testicular infiltration in 49% and ovarian infiltration in 58%. Reid et al. [25] autopsied patients with leukaemia and lymphoma and found testicular infiltration in 42/65 (65%) of cases and ovarian infiltration in 20/31 (66%) of cases. However, gonadal metastases have also been described in solid tumours such as neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma and carcinomas. The most frequent gonadal metastases are found in neuroblastoma and among sarcomas in

rhabdomyosarcoma [26–28]. Further data on the risk of gonadal metastasis in adults can be found in chapter "Removal of ovarian tissue" and in the review by Basting et al. [28].

Due to potential gonadal metastasis, retransplantation of cryopreserved gonadal tissue is not possible in many cases. Patients and their parents must be informed about this before tissue is removed. Techniques are currently being developed (see chapter "Further Fertility Preservation Techniques") which may also allow the tissue to be used in the event of metastasis. However, these are experimental, and it cannot yet be estimated whether they will be clinically useful at all, and if so, when.

Fertility-Preservation Methods

The fertility-preservation methods available for girls and boys before paediatric oncological therapy differ in whether the start of therapy is before or after puberty. In addition to established measures, experimental ones (especially in prepubertal children) are also available [20].

Fertility-Preservation Methods in Girls

GnRH Agonists (see Chapter "GnRH Agonists")

GnRH agonists are not indicated in children and their effectiveness in adolescents is questionable.

Ovarian Transposition (see Chapter "Transposition of Ovaries")

Depending on the radiation dose (see chapter "Pregnancy After Chemotherapy and Radiation of the Pelvis"), transposition should be considered if the ovaries are in the radiation field during radiotherapy, the benefit of which is proven if the ovaries can be luxated far enough out of the radiation field. In addition to known complications such as circulatory disorders and cysts in the displaced ovary, it should be noted that the fallopian tubes must be cut if the ovaries are transposed under the diaphragm, which makes in vitro fertilisation necessary at a later date. It must also be pointed out that, depending on the radiation dose, simultaneous irradiation may result in increased pregnancy risks or even make pregnancy impossible due to damage to the uterus (see chapter "Pregnancy After Chemotherapy and Radiation of the Pelvis").

Ovarian Stimulation and Freezing of Oocytes (see Chapters "Ovarian Stimulation to Collect Oocytes" and "Cryopreservation of Unfertilized and Fertilized Oocytes")

Due to the urgency of the oncological therapy, it is not always possible to carry out the hormonal stimulation required for about 14 days prior to the cryopreservation of oocytes. Such a measure is only feasible in adolescents with already activated folliculogenesis. It requires regular vaginal ultrasound examinations and transvaginal follicular puncture, which is only possible if the size of the hymenal opening allows such an examination. The psychological strain of vaginal manipulation must also be considered.

Removal and Freezing of Ovarian Tissue (see Chapters "Removal of Ovarian Tissue" and "Transportation, Cryopreservation and Storage of Ovarian Tissue")

Cryopreservation is possible, taking into consideration of the risks of gonadal metastasis (see above) and requires laparoscopy. Two case reports on puberty induction with retransplanted ovarian tissue cryopreserved in childhood [29, 30] and the report on a first birth after retransplantation of ovarian tissue removed at the beginning of puberty [31] show that tissue activation is possible after retransplantation. Since the later pregnancy rate after retransplantation of ovarian tissue with a high follicle density (as is the case with children) is particularly high, this measure appears to be well suited for children. However, the risk of retransplantation of malignant cells must be considered.

Fertility-Preservation Methods in Boys

Cryopreservation of Sperm (see Chapter "Cryopreservation of Sperm and Testicular Tissue")

Cryopreservation of sperm after puberty (ejaculation, electrostimulation, testicular biopsy [TESE]) as a fertility reserve for later assisted reproductive measures is possible from the age of 13, Tanner 3 and a testicular volume of $\geq$10 mL [32].

Cryopreservation of Immature Testicular Tissue (see Chapter "Cryopreservation of Sperm and Testicular Tissue")

The cryopreservation of immature testicular tissue surgically removed before puberty is still experimental. The necessary subsequent sperm maturation from the testicular stem cells is not yet possible in humans. Depending on the malignant disease, there is also a risk of retransplantation of malignant cells.

Practical Approach

The risk of gonadal damage should first be evaluated using the data in section "Gonadotoxicity of Therapies". As already described in this chapter, indications for or against a fertility preservation measure in children cannot always be clearly defined. However, if there is a high risk of gonadal toxicity, fertility-preservation measures are strongly recommended before starting gonadotoxic therapy. Fertility preservation should be discussed if there is a medium risk, and they can also be discussed if the risk is low. Young patients should be actively involved in the decision-making process.

The risk of a fertility disorder should be explained in detail to those affected and their relatives. The risks of fertility preservation methods must also be explained and later alternatives such as sperm and egg donation (if permitted in the country concerned) must be considered. If the pelvis is irradiated, the risks for a later pregnancy must be explained (see chapter "Pregnancy After Chemotherapy and Radiation of the Pelvis"). It is important to note that gonadotoxic treatments do not lead to an increased risk of malformations or non-hereditary cancer in the offspring [33–35].

After completion of the oncological therapy, care must be taken to ensure normal pubertal development. A fertility assessment should be carried out post-pubertally or in young adulthood at the latest.

References

1. Deutsches Kinderkrebsregister. Jahresbericht 2017. (2017). http://www.kinderkrebsregister.de/typo3temp/secure_downloads/22605/0/bd894cced68c7950a69875dc2588a29281fd6fbc/jb2017_s.pdf
2. von Wolff M, Dittrich R, Liebenthron J, Nawroth F, Schüring AN, Bruckner T, Germeyer A. Fertility-preservation counselling and treatment for medical reasons: data from a multinational network of over 5000 women. Reprod Biomed Online. 2015;31:605–12. https://doi.org/10.1016/j.rbmo.2015.07.013.
3. Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, Donaldson SS, Byrne J, Robison LL. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol. 2009;27:2677–85. https://doi.org/10.1200/JCO.2008.20.1541.
4. Rendtorff R, Hohmann C, Reinmuth S, Müller A, Dittrich R, Beyer M, Wickmann L, Keil T, Henze G, Borgmann-Staudt A. Hormone and sperm analyses after chemo- and radiotherapy in childhood and adolescence. Klin Padiatr. 2010;222:145–9. https://doi.org/10.1055/s-0030-1249658.
5. Balcerek M, Reinmuth S, Hohmann C, Keil T, Borgmann-Staudt A. Suspected infertility after treatment for leukemia and solid tumors in childhood and adolescence. Dtsch Arztebl Int. 2012;109:126–31. https://doi.org/10.3238/arztebl.2012.0126.
6. Borgmann-Staudt A, Rendtorff R, Reinmuth S, Hohmann C, Keil T, Schuster FR, Holter W, Ehlert K, Keslova P, Lawitschka A, Jarisch A, Strauss G. Fertility after allogeneic haematopoietic stem cell transplantation in childhood and adolescence. Bone Marrow Transplant. 2012;47:271–6. https://doi.org/10.1038/bmt.2011.78.
7. Pfitzer C, Orawa H, Balcerek M, Langer T, Dirksen U, Keslova P, Zubarovskaya N, Schuster FR, Jarisch A, Strauss G, Borgmann-Staudt A. Dynamics of fertility impairment in childhood brain tumour survivors. J Cancer Res Clin Oncol. 2014;140:1759–67. https://doi.org/10.1007/s00432-014-1702-7.

8. Hohmann C, Borgmann-Staudt A, Rendtorff R, Reinmuth S, Holzhausen S, Willich SN, Henze G, Goldbeck L, Keil T. Patient counselling on the risk of infertility and its impact on childhood cancer survivors: results from a national survey. J Psychosoc Oncol. 2011;29:274–85. https://doi.org/10.1080/07347332.2011.563344.
9. Hohmann C, Borgmann A, Keil T. Re: induced abortions in Danish cancer survivors: a population based cohort study. J Natl Cancer Inst. 2011;103:698. https://doi.org/10.1093/jnci/djr063.
10. Reinmuth S, Hohmann C, Rendtorff R, Balcerek M, Holzhausen S, Müller A, Henze G, Keil T, Borgmann-Staudt A. Impact of chemotherapy and radiotherapy in childhood on fertility in adulthood: the FeCt-survey of childhood cancer survivors in Germany. J Cancer Res Clin Oncol. 2013;139:2071–8. https://doi.org/10.1007/s00432-013-1527-9.
11. Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol. 2005;6:209–18.
12. Wasilewski-Masker K, Seidel KD, Leisenring W, Mertens AC, Shnorhavorian M, Ritenour CW, Stovall M, Green DM, Sklar CA, Armstrong GT, Robison LL, Meacham LR. Male infertility in long-term survivors of pediatric cancer: a report from the childhood cancer survivor study. J Cancer Surviv. 2014;8:437–47. https://doi.org/10.1007/s11764-014-0354-6.
13. Brämswig JH, Heimes U, Heiermann E, Schlegel W, Nieschlag E, Schellong G. (1990) The effects of different cumulative doses of chemotherapy on testicular function. Results in 75 patients treated for Hodgkin's disease during childhood or adolescence. Cancer 65:1298-1302.
14. AWMF. S3 Leitlinie 025-030. Endokrinologische Nachsorge nach onkologischen Erkrankungen im Kindes- und Jugendalter. . http://www.awmf.org/uploads/tx_szleitlinien/025-030l_S3_Endokrinologische_Nachsorge_nach_onkologischen_Erkrankungen_Kindes_Jugendalter_2014_03.pdf
15. Jahnukainen K, Ehmcke J, Hou M, Schlatt S. Testicular function and fertility preservation in male cancer patients. Best Prac Res Clin Endocrinol Metab. 2011;25:287–302. https://doi.org/10.1097/MED.0000000000000156.
16. Wei C, Crowne E. The impact of childhood cancer and its treatment on puberty and subsequent hypothalamic pituitary and gonadal function, in both boys and girls. Best Pract Res Clin Endocrinol Metab. 2019;33(3):101291. https://doi.org/10.1016/j.beem.2019.101291.
17. Wallace WH, Thomson AB, Sarana F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. Int J Radiot Oncol Bio Biol Phys. 2005;62:738–44.
18. Dörr W, Herrmann T. Prophylaxe von Fertilitätsstörungen nach Strahlentherapie. Focus Onkologie. 2005;9:58–60.
19. AWMF. S1 Leitlinie 027-025. Pubertas tarda. . http://www.awmf.org/uploads/tx_szleitlinien/027-025l_S1_Pubertas_tarda_Hypogonadismus_2011-01.pdf
20. Wessel T, Balcerek M, Reinmuth S, Hohmann C, Keil T, Henze G, Borgmann-Staudt A. Age at menarche in childhood cancer survivors: results of a nationwide survey in Germany. Horm Res Paediatr. 2012;77:108–14. https://doi.org/10.1159/000336688.
21. Koustenis E, Pfitzer C, Balcerek M, Reinmuth S, Zynda A, Stromberger C, Hohmann C, Keil T, Borgmann-Staudt A. Impact of cranial irradiation and brain tumor location on fertility: a survey. Klin Padiatr. 2013;225:320–4. https://doi.org/10.1055/s-0033-1353206.
22. AWMF S1 Leitlinie 025-034. Beeinträchtigung der Gonadenfunktion nach Chemo- und Strahlentherapie im Kindes- und Jugendalter: Risiken, Diagnostik, Prophylaxe- und Behandlungsmöglichkeiten. http://www.awmf.org/uploads/tx_szleitlinien/025-034_l_S1_Beeinträchtigung_Gonadenfunktion_nach_Krebs_im_Kindesalter_2015-03.pdf
23. Howell SJ, Shalet SM. Effect of cancer therapy on pituitary-testicular axis. Int J Androl. 2002;25:269–76.
24. Kamiyama R, Funata N. A study of leukemic cell infiltration in the testis an ovary. Bull Tokyo Med Dent Univ. 1976;23:203–10.
25. Reid H, Marsden HB. Gonadal infiltration in children with leukaemia and lymphoma. J Clin Pathol. 1980;33:722–9.
26. Somjee S, Kurkure PA, Chinoy RF, Deshpande RK, Advani SH. Metastatic ovarian neuroblastoma: a case report. Pediatr Hematol Oncol. 1999;16:459–62.

27. Young RH, Kozakewich HP, Scully RE. Metastatic ovarian tumors in children: a report of 14 cases and review of the literature. Int J Gynecol Pathol. 1993;12:8–19.
28. Bastings L, Beerendonk CC, Westphal JR, Massuger LF, Kaal SE, van Leeuwen FE, Braat DD, Peek R. Autotransplantation of cryopreserved ovarian tissue in cancer survivors and the risk of reintroducing malignancy: a systematic review. Hum Reprod Update. 2013;19:483–506. https://doi.org/10.1093/humupd/dmt020.
29. Poirot C, Abirached F, Prades M, Coussieu C, Bernaudin F, Piver P. Induction of puberty by autograft of cryopreserved ovarian tissue. Lancet. 2012;37:588. https://doi.org/10.1016/S0140-6736(11)61781-9.
30. Ernst E, Kjaersgaard M, Birkebaek NH, Clausen N, Andersen CY. Case report: stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. Eur J Cancer. 2013;49:911–4. https://doi.org/10.1016/j.ejca.2012.09.028.
31. Demeestere I, Simon P, Dedeken L, Moffa F, Tsépélidis S, Brachet C, Delbaere A, Devreker F, Ferster A. Live birth after autograft of ovarian tissue cryopreserved during childhood. Hum Reprod. 2015;30:2107–9. https://doi.org/10.1093/humrep/dev128.
32. Nielsen CT, Skakkebaek NE, Richardson DW, Darling JA, Hunter WM, Jørgensen M, Nielsen A, Ingerslev O, Keiding N, Müller J. Onset of the release of spermatozoa (spermarche) in boys in relation to age, testicular growth, pubic hair, and height. J Clin Endocrinol Metab. 1986;62:532–5.
33. Nagarajan R, Robison LL. Pregnancy outcomes in survivors of childhood cancer. J Natl Cancer Inst Monogr. 2005;2005:72–6.
34. Winther JF, Boice JD Jr, Frederiksen K, Bautz A, Mulvihill JJ, Stovall M, Olsen JH. Radiotherapy for childhood cancer and risk for congenital malformations in offspring: a population-based cohort study. Clin Genet. 2009;75:50–6. https://doi.org/10.1111/j.1399-0004.2008.01109.x.
35. Winther JF, Boice JD Jr, Christensen J, Frederiksen K, Mulvihill JJ, Stovall M, Olsen JH. Hospitalizations among children of survivors of childhood and adolescent cancer: a population-based cohort study. Int J Cancer. 2010;127:2879–87. https://doi.org/10.1002/ijc.25286.

Other Malignancies

Michael von Wolff and Martin F. Fey

Introduction

About 40% of the women who presented to the *Ferti*PROTEKT network for fertility preservation therapy in 2013–2018 were treated for breast cancer and 17% for Hodgkin's lymphoma. There were also many consultations regarding non-Hodgkin's lymphoma, ovarian tumours and leukaemia (approx. 4% each). The remaining 20% of the consultations were distributed among ca. 200 further diagnoses (Table 1).

Part II of this book deals with illnesses which make up about 70% of consultations. This chapter deals with all diseases and disease groups which are documented with a frequency of at least 1:500 and which have not already been discussed in detail in Part II (12% of consultations, (Tables 2–9)). Therefore, diseases from ca. 20% of consultations cannot be described due to their wide variety. Data on these cases must be researched individually during a consultation.

This chapter only deals with the diseases in tabular form. The tables can only give a rough orientation for the reproductive physician. Further information from each patient must be included and evaluated individually.

Regarding gonadotoxicity data, the following applies as a rough guide:

- *Low*: <ca. 20%: no fertility preservation therapy recommended.
- *Medium*: >20%: consider/recommend fertility preservation therapy.

M. von Wolff (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. F. Fey
University Clinic for Medical Oncology, University of Bern, Bern, Switzerland
e-mail: Martin.Fey@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_13

Table 1 Diseases and number of consultations in the *Ferti*PROTEKT network during 2013–2018 [1]

Total number of consultations, *n*	6664		
Breast cancer, *n*	2695	40.4%	Chapter "Breast Cancer"
Hodgkin's lymphoma, *n*	1138	17.1%	Chapter "Hodgkin's Lymphoma"
Ovarian tumours, *n*	271	4.1%	Chapter "Ovarian Tumours and Ovarian Cancer"
Non-Hodgkin's lymphoma, *n*	262	3.9%	Table 2
Acute leukaemia, *n*	236	3.5%	Chapter "Acute Leukaemia"
Brain tumours, *n*	128	1.9%	Table 3
Colorectal and anal cancers, *n*	124	1.9%	Table 4
Rheumatic disease, *n*	97	1.6%	Chapter "Severe Autoimmune Diseases"
Ewing's sarcoma, *n*	93	1.4%	Table 5
Osteosarcoma, *n*	84	1.3%	Table 6
Benign haematological diseases, *n*	80	1.2%	Chapter "Non-Malignant Diseases Requiring Stem Cell Transplantation"
Soft tissue including liposarcoma, *n*	42	0.6%	Table 7
Stomach cancer, *n*	30	0.5%	Table 8
Turner syndrome, *n*	29	0.4%	Chapter "Turner Syndrome"
Endometriosis, *n*	19	0.3%	Chapter "Endometriosis"
Endometrial cancer, *n*	13	0.2%	Chapter "Endometrial Hyperplasia and Endometrial Carcinoma"
Other diseases, *n*	1323	19.9%	Not described

Only diseases and disease groups with a consultation frequency of ≥1:500 (≥0.02%) are listed. The number of consultations/diseases, the relative frequency in relation to all registered consultations and the chapters in which the illnesses are treated are shown. The diseases without chapter reference are outlined in tabular form in this chapter

- *High*: fertility therapy urgently recommended (predominant amenorrhoea/azoospermia).

Additional reference is made to Table 2 of chapter "Removal of ovarian tissue" regarding the risk of ovarian metastases.

Table 2 Non-Hodgkin's lymphoma

Incidence	5-year survival rate	Gonadotoxicity of the oncological treatment	Risk of ovarian metastasis	Recommended fertility preservation measures with curative therapy approach
New cases approx. 20/100,000, more likely in older age *Highly malignant lymphomas* (blast forms and Burkitt's lymphomas): rapidly progressive, cure possible, immediate therapy *Low-grade lymphomas:* slowly progressive, therapy only when symptoms appear	All stages: 65% *Poor prognostic factors:* Age >60 years, poor general health, increased LDH concentration, >1 extranodal manifestation, stage III or IV *High-grade malignant NHL:* if 4–5 poor prognostic factors: 50%, if no poor prognostic factors: high cure rate *Low-grade malignant NHL:* only really curable in stages I–II, in the frequent stages III–IV progress often long and indolent	*Medium:* for low-grade malignant forms: e.g. chemotherapy regimen R-CHOP, R-COP [2, 3] *Medium/high:* for high-grade malignant forms: therapy similar to leukaemias see chapter "Acute Leukaemia"	*High:* in blastocytic forms and Burkitt's lymphomas (similar to leukaemias, see chapter "Acute Leukaemia") *Medium:* for other non-Hodgkin's lymphomas Examination of ovarian tissue: Briseno-Hernandez et al. [4]: one woman: evidence of Burkitt's lymphoma in both ovaries. Meirow et al. [5]: 2 women: ovarian metastases detected by imaging, therefore no cryopreservation of ovarian tissue, 14 women: histological examination of ovarian tissue: no tumour detection. Hoekmann et al. [6]: 2 women: No tumour detection. Dolmanns et al. [7]: malignant cells in 2/26 women.	*High-grade malignant, blastocytic and Burkitt's lymphomas:* see chapter "Acute Leukaemia" *Low-grade malignant forms:* Individual decision depending on therapy: Women: GnRH agonists, cryopreservation of oocytes after stimulation, cryopreservation of ovarian tissue with reservations due to the risk of ovarian metastasis Men: Cryopreservation of sperm, Cryopreservation of testicular tissue with reservations due to the risk of testicular metastasis

Table 3 Brain tumour—glioma

Incidence	5-year survival rate	Effect of oncological therapy on fertility	Risk of ovarian metastasis	Recommended fertility preservation measures with curative therapy approach
New cases: 6/100,000 *WHO grade I:* pilocytic astrocytoma: low malignant tumour in childhood *WHO grade II:* Astrocytoma, oligo-dendroglioma, oligo-astrocytoma *WHO grade III:* anaplastic astrocytoma *WHO grade IV:* glioblastoma	*Five-year survival rate:* all forms: 40–50% Prognosis depends, among other things, on whether a mutation of isocitrate dehydrogenase (IDH) is present (IDHmut) or not (wild type, IDHwt) *Average survival:* *WHO II:* IDHmut: 10 years IDHwt: up to 3 years. *WHO III:* IDHmut: 6–8 years, IDHwt: 1–4 years *WHO IV:* 14–23 months	*WHO I:* only local surgical therapy, no effect on fertility *WHO II-IV:* mostly surgery plus radiochemotherapy Radiotherapy [8]: Post-pubertal irradiation of the hypothalamus and pituitary gland with 39–70 Gy (mean 53 Gy) resulted in oligomenorrhea in 70% and hyperprolactinemia in 50%. Radiotherapy to the hypothalamus seems to be particularly relevant. In prepubertal girls (30 Gy) tendency is only premature puberty and shortened luteal phases Chemotherapy: Temozolomide (low risk) or procarbazine (medium risk)	Low	*If chemotherapy with temozolomide:* No fertility preservation required. *If chemotherapy with procarbazine:* Women: GnRH agonists, cryopreservation of oocytes, Cryopreservation of ovarian tissue Men: Cryopreservation of sperm, possibly cryopreservation of testicular tissue *Radiotherapy-induced hypothalamic-pituitary infertility*: Treatable with gonadotropins if wish to get pregnant

Table 4 Colorectal and anal cancer

Incidence	5-year survival rate	Gonadotoxicity of the oncological treatment	Risk of ovarian metastasis	Recommended fertility preservation measures with curative therapy approach
Colorectal cancer: New cases: approx. 20–40/100,000 (30% rectum, 70% colon*)* *Anal cancers:* 1/100,000, tends to be in older age	*Colorectal cancer:* *After curative resection (pR0):* Colon cancer: 70–80% Rectal cancer: 60–70%, *depending on the tumour stage (pT1–3) for R0 resections:* 90, 80, 60% (colon) 90, 70, 40% (rectum) *Anal cancer:* 80%—if treatable with radiochemotherapy only. 30–60%—if additional rectal amputation is required.	*Curative adjuvant treatment* *Low:* if chemotherapy with 5-fluorouracil/capecitabine *low/medium*: Combinations with oxaliplatin (colorectal carcinoma), mitomycin-C (only anal cancer) *High:* if pelvic radiotherapy including the ovaries (see chapter "Indications for and Against Fertility Preservation") (rectal and anal cancer) Cave: High-dose irradiation of the uterus is not compatible with a later pregnancy (see chapter "Pregnancy After Chemotherapy and Radiation of the Pelvis"). In cases of metastatic colorectal cancer, palliative chemotherapy with oxaliplatin or irinotecan and various antibodies: moderate gonadal toxicity	*Low:* if stage I–III Examination of ovarian tissue: Hoeckmann et al. [6]: one woman with rectal cancer: no tumour detection	Women: *With pelvic/ovarian radiotherapy:* Transposition of the ovaries *If chemotherapy:* GnRH agonists If medium/high risk of gonadal toxicity: Cryopreservation of oocytes, cryopreservation of ovarian tissue only if no pelvic radiotherapy Men: If medium/high risk of gonadal toxicity: Cryopreservation of sperm, possibly cryopreservation of testicular tissue

Table 5 Ewing's sarcoma

Incidence	5-year survival rate	Gonadotoxicity of the oncological treatment	Risk of ovarian metastasis	Recommended fertility preservation measures with curative therapy approach
New cases: 0.3/100,000 children <15 years, 0.24/100,000 adolescents and young adults	*Risk category "Standard":* 70–75%: localized tumour and initial tumour volume ≤200 mL and good histological response to neoadjuvant chemotherapy *Risk category "high":* *approximately 50%:* localized tumour and initial tumour volume >200 mL or poor histological response to neoadjuvant chemotherapy or pulmonary metastases as the only localisation of metastasis *Risk category "very high":* Approximately 20–40%: All others	*Medium:* If chemotherapy only *High:* If in combination with pelvic radiotherapy or autologous stem cell transplantation [9] *Cave:* High-dose irradiation of the uterus is not compatible with a later pregnancy (see chapter "Pregnancy After Chemotherapy and Radiation of the Pelvis").	*Possible in individual cases* Examination of ovarian tissue: Greve et al. [10]: 9 women: no tumour detection. Abir et al. [11]: 8 women, 7 women without, one woman with tumour detection. Hoekmann et al. [6]: 4 women: no tumour detection. Dolmans et al. [12]: 14 women: no tumour detection. Sörensen et al. [13] (predominantly imaging but not histological diagnosis): 514 women in 3 studies: one woman with ovarian metastasis.	Women: GnRH agonists, cryopreservation of oocytes, cryopreservation of ovarian tissue Men: Cryopreservation of sperm, possibly cryopreservation of testicular tissue

Table 6 Osteosarcoma

Incidence	5-year survival rate	Gonadotoxicity of the oncological treatment	Risk of ovarian metastasis	Recommended fertility preservation measures with curative therapy approach
New cases: ca. 0.2–0.3/100,000	*Classic central (highly malignant) osteosarcoma:* Treated only surgically: maximum 20%; with (neo-) adjuvant chemotherapy: 50–70% *Parosteal osteosarcoma:* Low-grade malignancy in 70–80% of cases; 80% after adequate surgery	Medium: Chemotherapy: POI in 6/90 young women [14]	*Possible in individual cases* Examination of ovarian tissue: Greve et al. [10]: 4 women: no tumour detection. Hoekmann et al [6]: 7 women: no tumour detection.	Women: GnRH agonists, cryopreservation of oocytes, cryopreservation of ovarian tissue Men: Cryopreservation of sperm, possibly cryopreservation of testicular tissue

Table 7 Soft tissue and liposarcoma

Incidence	5-year survival rate	Gonadotoxicity of the oncological treatment	Risk of ovarian metastasis	Recommended fertility preservation measures with curative therapy approach
Soft tissue sarcoma: ca. 2–3/100,000 *Liposarcoma:* ca. 0.25/100,000	*Soft tissue sarcoma:* Stages I, II, III: 99%, 82%, 52% *Liposarcoma:* well differentiated: almost 100%, myxoid: 88%, poorly differentiated or round cell: 50%	Primary treatment: surgical removal, if necessary additional: radio- and/or chemotherapy. Gonadotoxicity depends on chemotherapy and localisation of radiotherapy	*Possible in individual cases* Examination of ovarian tissue: Greve et al. [10]: 3 women: no tumour detection. Dolmans et al. [12]: 12 women: no tumour detection	Women: GnRH agonists, cryopreservation of oocytes, cryopreservation of ovarian tissue Men: Cryopreservation of sperm, possibly cryopreservation of testicular tissue

Table 8 Stomach cancer

Incidence	5-year survival rate	Gonadotoxicity of the oncological treatment	Risk of ovarian metastasis	Recommended fertility preservation measures with curative therapy approach
11/100,000, tends to be in older age	All stages: ca. 30% Early stomach cancer, stage I (limited to mucosa and submucosa, individual lymph node metastases possible): >80%	No gonadotoxicity: Stage IA as surgical therapy only Low/medium: Stage IB–III: Surgery plus (mostly preoperative) platinum-containing chemotherapy	Low: if stage I–III	Only with good prognosis and gonadotoxic chemotherapy: Women: GnRH agonists, cryopreservation of oocytes, cryopreservation of ovarian tissue Men: Cryopreservation of sperm, possibly cryopreservation of testicular tissue

Table 9 Testicular cancer

Incidence	5-year survival rate	Gonadotoxicity of the oncological treatment	Recommended fertility preservation measures with curative therapy approach
8–10/100,000 *Seminoma:* 40–55% *Non-seminoma:* 45–60%	*Seminoma:* If no remote metastases: 90% If remote metastases: 70–80% *Non-seminoma:* If no remote metastases and tumour markers only slightly elevated: 90% If no remote metastases and tumour markers moderately increased: 70–80% If remote metastases or tumour marker greatly increased: 50%.	Medium: After chemo and/or radiotherapy, 207 men tried to father a child: 77% successful, 5% after infertility treatment, 18% unsuccessful (female sterility factors not taken into account) [15]. Sperm quality often reduced due to illness	In post pubertal males: Cryopreservation of sperm or in individual cases of testicular tissue (TESE) In prepubertal males: cryopreservation of testicular tissue with special medium for obtaining spermatogonia (see chapter “Cryopreservation of Sperm and Testicular Tissue”)

References

1. Germeyer A, Dittrich R, Liebenthron J, Nawroth F, Sänger N, Suerdieck M, et al. FertiPROTEKT-Auswertungen 2018. J Reproduktionsmed Endokrinol. 2019;16:41–4.
2. Elis A, Tevet A, Yerushalmi R, Blickstein D, Bairy O, Dann EJ, et al. Fertility status among women treated for aggressive non-Hodgkin's lymphoma. Leuk Lymphoma. 2006;47:623–7.
3. Gini G, Annibali O, Lupasco D, Bocci C, Tomarchio V, Sampaolo M, et al. Gonadal function recovery and fertility in women treated with chemo- and/or radiotherapy for Hodgkin's and Non-Hodgkin lymphoma. Chemotherapy. 2019;64:36–41.
4. Briseño-Hernández AA, Quezada-López DR, Castañeda-Chávez A, Dassaejv Macías-Amezcua M, Pintor-Belmontes JC. Bilateral ovarian Burkitt's lymphoma. A case presentation. Cir Cir. 2014;82:212–8.
5. Meirow D, Hardan I, Dor J, Fridman E, Elizur S, Ra'anani H, et al. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. Hum Reprod. 2008;23:1007–13.
6. Hoekman EJ, Smit VT, Fleming TP, Louwe LA, Fleuren GJ, Hilders CG. Searching for metastases in ovarian tissue before autotransplantation: a tailor-made approach. Fertil Steril. 2015;103:469–77.
7. Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, et al. A review of 15 years of ovarian tissue bank activities. J Assist Reprod Genet. 2013;30:305–14.
8. Wo JY, Viswanathan AN. Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients. Int J Radiat Oncol Biol Phys. 2009;73:1304–12.
9. Raciborska A, Bilska K, Filipp E, Drabko K, Rogowska E, Chaber R, et al. Ovarian function in female survivors after multimodal Ewing sarcoma therapy. Pediatr Blood Cancer. 2015;62:341–5.
10. Greve T, Wielenga VT, Grauslund M, Sørensen N, Christiansen DB, Rosendahl M, et al. Ovarian tissue cryopreserved for fertility preservation from patients with Ewing or other sarcomas appear to have no tumour cell contamination. Eur J Cancer. 2013;49:1932–8.
11. Abir R, Feinmesser M, Yaniv I, Fisch B, Cohen IJ, Ben-Haroush A, et al. Occasional involvement of the ovary in Ewing sarcoma. Hum Reprod. 2010;25:1708–12.
12. Dolmans MM, Iwahara Y, Donnez J, Soares M, Vaerman JL, Amorim CA, Poirel H. Evaluation of minimal disseminated disease in cryopreserved ovarian tissue from bone and soft tissue sarcoma patients. Hum Reprod. 2016;31:2292–302.
13. Sørensen SD, Greve T, Wielenga VT, Wallace WH, Andersen CY. Safety considerations for transplanting cryopreserved ovarian tissue to restore fertility in female patients who have recovered from Ewing's sarcoma. Future Oncol. 2014;10:277–83.
14. Longhi A, Pignotti E, Versari M, Asta S, Bacci G. Effect of oral contraceptive on ovarian function in young females undergoing neoadjuvant chemotherapy treatment for osteosarcoma. Oncol Rep. 2003;10:151–5.
15. Huddart RA, Norman A, Moynihan C, Horwich A, Parker C, Nicholls E, Dearnaley DP. Fertility, gonadal and sexual function in survivors of testicular cancer. Br J Cancer. 2005;93:200–7.

Non-Malignant Diseases Requiring Stem Cell Transplantation

Andrea Jarisch and Ariane Germeyer

Introduction

Haematopoietic stem cell transplantation (HSCT) is an established and often the only curative treatment for serious haematological diseases. The number of stem cell transplantations for non-malignant diseases has therefore continuously increased over the last decades and has led to better overall survival of patients with congenital and acquired non-malignant diseases [1–3]. Advances in transplantation medicine and improved possibilities for diagnosis and thus indications for HSCT are responsible for this trend. With this development, topics such as quality of life and long-term consequences of HSCT are increasingly common, because the conditioning required for HSCT leads to infertility in 80–100% of patients, depending on the conditioning protocol used [4, 5].

Many studies highlight the importance of fertility for the quality of life of long-term surviving patients. The frequency of patients wishing to have a child after HSCT corresponds with that of the normal population [6]. Many HSCTs in patients with non-malignant diseases are diagnosed in early childhood, i.e. long before puberty. Fortunately, advances in reproductive medicine are opening new perspectives, especially for prepubertal patients, through the use of fertility-preserving measures [7]. An overview of the most common non-malignant diseases that can be treated by HSCT is shown in Table 1.

A. Jarisch (✉)
Division of Stem Cell Transplantation and Immunology, Department for Children and Adolescents, University Hospital, Goethe University, Frankfurt am Main, Germany
e-mail: andrea.jarisch@kgu.de

A. Germeyer
Department of Gynaecological Endocrinology and Fertility Disorders, University Women's Hospital Heidelberg, Heidelberg, Germany
e-mail: ariane.germeyer@med.uni-heidelberg.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_14

Table 1 Non-malignant diseases treatable by haematopoietic stem cell transplantation (HSCT) (modified [27])

Main groups of hereditary disorders	Specific hereditary disorders
Red cell disorders	
	Thalassaemia major
	Sickle cell disease
	Congenital erythropoietic porphyria (CEP, Gunther's disease)
	Congenital dyserythropoietic anaemia (CDA, type I and II)
Bone marrow failure	
Pancytopenia	Fanconi anaemia
	Shwachman–Diamond syndrome
	Dyskeratosis congenita
Red cell aplasia	Diamond–Blackfan anaemia
Neutropenia	Severe congenital neutropenia
Platelet disorders	Congenital amegakaryocytic thrombocytopenia
Haemophagocytic conditions	
	Haemophagocytic lymphohistiocytosis (HLH)
	Criscelli syndrome
	Chediak–Higashi syndrome
Immunodeficiency	
Severe combined immunodeficiency (SCID)	SCID +/− B/− T-cells ADA-deficient SCID
Non-SCID immunodeficiency diseases	Omenn's syndrome
	Wiskott–Aldrich syndrome
	CD40 ligand deficiency
	Leukocyte adhesion deficiency syndrome (LAD)
	Chronic granulomatous disease (CGD)
	X-linked lymphoproliferative disease
Metabolic disease	
	Hurler syndrome
	X-linked adrenoleukodystrophy (ALD)
	Metachromatic leukodystrophy (MLD)
	Malignant infantile Osteopetrosis
Other haematological conditions	
	Severe aplastic anaemia (SAA)
	Myelodysplastic syndrome (MDS), type refractory cytopenia (RC)

Treatment Gonadotoxicity

Hematopoietic Stem Cell Transplantation

Gonadotoxic effects of chemotherapy and radiotherapy are well known [8–10]. Alkylating drugs in particular lead to partial or complete damage of gonadal

function in both sexes, with the possible loss of germ cells or a shortened reproductive phase in affected girls and women [10, 11].

The risk of infertility after HSCT depends on the underlying disease, a reduced ovarian reserve even before the start of treatment, previous treatments, the conditioning drugs used and the age of the patient at the time of HSCT [5, 8, 10].

Only 1–5% of all patients who have undergone stem cell transplantation have children. However, there is little published data from adults who underwent transplantation as children or adolescents [5, 10]. Some studies report higher residual fertility when HSCT is performed at a younger age [8].

Regarding the regimes used, cyclophosphamide monotherapy showed the lowest gonadotoxic effect [11]. However, this protocol is only used in patients with severe aplastic anaemia. Patients who received busulfan-based protocols or total body irradiation (TBI) had a birth rate of <1% [5, 10–12].

Paediatric conditioning protocols are often myeloablative (87%); however, there is a trend to achieve conditioning with reduced intensity or toxicity (from 8% in 2000 to 16% in 2015) [4]. These protocols are preferably used in non-malignant settings. Further long-term studies are necessary to prove the suspected reduced gonadotoxic effect of these protocols [13, 14]. Two pregnancies were recently reported in a woman treated with a reduced intensity protocol at the age of 19 years [15].

Due to the lack of data on fertility after conditioning with reduced toxicity, all patients receiving HSCT after conditioning should currently be advised on fertility preservation measures.

Gene Therapy

In 2018, the first marketing authorisation application for gene therapy for patients with thalassaemia major was submitted to the European Medicines Agency (EMA). The EMA recommends approval of the new gene therapy for thalassaemia patients and the application is expected to be implemented in 2020.

Immunodeficiencies, metabolic disorders and cystic fibrosis are also among the diseases that are potentially eligible for gene therapy. Before retransfusion of the autologous genetically modified stem cells, conditioning, currently mostly with busulfan, is necessary. These patients should also be advised on the gonadotoxic effect of conditioning and fertility-preserving measures should be offered.

Advice on Fertility Preservation Measures

In 2016, experts from the Paediatric Diseases Working Party (PDWP) of the European Society for Blood and Marrow Transplantation (EBMT) published recommendations for advice and implementation of fertility-preserving measures in children and adolescents undergoing planned HSCT [3]. All patients of prepubertal

or postpubertal age should be offered advice on the options available to them for fertility preservation. Counselling should include information on the risk of fertility impairment as a result of planned HSCT in relation to the underlying disease, age, pre-treatment and other comorbidities. At the request of patients or their parents, an individualised concept for maintaining fertility should be an integral part of the treatments.

During the consultation, attention should be drawn to the possibility of inheriting the underlying disease, especially in the case of autosomal dominant or x-linked inherited diseases. It is essential that patients are informed about the possible inheritance of these defective genes to their offspring, even though the disease has been clinically cured after HSCT. Testing of the partner and consultation prior to initiating infertility treatment is essential. The implementation of fertility-preserving measures in mentally retarded patients should be discussed and considered in detail with the parents.

The currently still experimental character of fertility preservation methods in prepubertal girls should be pointed out. However, there are now first case reports of successful pregnancies after retransplantation of prepubertal ovarian tissue, so that this method may lose its experimental character in the coming years [7].

Restrictions on Fertility Preservation Measures in Special Situations

Patients with Thrombocytopenia/Neutropenia

Patients who have thrombocytopenia or neutropenia due to their underlying disease have an increased risk of bleeding or infection when gonadal tissue or oocytes are removed. Preoperative preventive measures, such as necessary substitutions, should be taken accordingly.

Patients with Sickle Cell Disease (SCD)

Many patients with SCD receive hydroxycarbamide (HC) as long-term treatment. Hydroxycarbamide is indicated for the prevention of recurrent painful vaso-occlusive crises including acute chest syndrome in adults, adolescents and children over 2 years of age with symptomatic SCD [16]. In a randomised, placebo-controlled trial, the BABY HUG study has shown that hydroxycarbamide administration also reduces the incidence of complications in previously asymptomatic infants [17].

Many guidelines recommend the early use of HC. Therefore, an increased proportion of SCD patients who received previous HC before allogeneis HSCT is to be expected. Boys and men, regardless of whether they were treated with HC, have

reduced spermatogenesis. There is a current lack of prospective studies on the negative influence of HC on fertility in girls and women [18]. However, a retrospective study showed a reduction in AMH concentration after HC therapy alone, and a negative influence on the ovarian reserve must currently be assumed [19].

Based on current data, it is not recommended that HC therapy be discontinued before fertility preservation measures are implemented, and a washout phase prior to conception should be considered for later transplantation of ovarian tissue obtained under HC therapy. Erythrocytes of patients with SCD have a shortened survival time and can lead to recurrent vascular occlusion crises and thromboses due to endothelial damage.

Cryopreservation of oocytes (see chapter "Cryopreservation of Unfertilized and Fertilized Oocytes") is an established procedure which requires hormonal stimulation (see chapter "Ovarian Stimulation to Collect Oocytes"). This can lead to complications in SCD patients, such as severe pain crises due to increased estrogen levels during stimulation and the occurrence of thromboses, especially acute thoracic syndrome or central nervous system infarctions. Case reports on complications after stimulation for oocyte collection have been published [20–22].

Some European stem cell transplant centres therefore prefer the removal and cryopreservation of ovarian tissue (personal communication) (see chapters "Removal of Ovarian Tissue" and "Transportation, Cryopreservation and Storage of Ovarian Tissue") as a fertility-preserving measure in pre- as well as post-pubertal SCD patients, in order to avoid hormonal stimulation.

Due to the lack of data and the possible serious complications associated with hormonal stimulation, we recommend not using hormonal stimulation and only offering these patients removal of ovarian tissue.

Recommendations regarding the perioperative procedure in SCD patients should be followed [23] for the removal of ovarian tissue (see chapter "Removal of Ovarian Tissue").

Patients with Iron Overload

Patients who receive regular red blood cell transfusions may develop endocrinopathies such as primary or secondary hypogonadism as a result of transfusion-related iron overload. Haemosiderosis-related endocrinopathies are more common in patients with thalassemia than with SCD [24]. Transfusion-dependent thalassaemia patients have ovulation disorders and hypogonadism in 30–80% of cases [25]. These changes are dependent, for example, on genotype and the start and duration of iron overload as a result of non-compliant chelation therapy [26]. The extent of iron-related damage to the pituitary gland and/or ovarian tissue can only be estimated approximately by ultrasound determination of the antral follicle count (AFC) and AMH concentration. If the ovarian reserve is demonstrably limited due to iron overload (AMH and AFC reduced), neither ovarian stimulation nor cryopreservation of ovarian tissue is appropriate.

Other Patients

Both ovarian stimulation and cryopreservation of ovarian tissue can be considered in all other postpubertal patients. In non-malignant situations, multiple stimulation cycles to achieve the desired number of cryopreserved oocytes may be possible.

Practical Approach

While possible fertility-preserving measures may be limited by the time frame available in patients with oncological diseases (e.g. myelodysplastic syndrome, MDS), patients with non-malignant diseases usually have sufficient time for a detailed consultation and implementation of fertility-preserving therapies (even repeated ovarian stimulation, if the underlying disease allows). An exception may be children with severe combined immunodeficiency (SCID) or haemophagocytic lymphohistiocytosis (HLH), whose diagnosis may only be made during a life-threatening and intensive exacerbation of the disease. In these exceptional cases, fertility-preserving measures should be avoided after risk assessment, but the parents should nevertheless be advised and involved in the decision-making process.

Fertility preservation measures can often be carried out during other planned surgical interventions, such as the implantation of an indwelling catheter or the removal of an autologous bone marrow reserve (autologous back-up).

References

1. European Society for Blood and Marrow Transplantation. Annual report, 2017. Leiden: EBMT; 2017.
2. Passweg JR, Baldomero H, Peters C, Gaspar HB, Cesaro S, Dreger P, Duarte RF, Falkenburg JH, Farge-Bancel D, Gennery A, Halter J, Kröger N, Lanza F, Marsh J, Mohty M, Sureda A, Velardi A, Madrigal A. European Society for Blood and Marrow Transplantation EBMT. Hematopoietic SCT in Europe: data and trends in 2012 with special consideration of pediatric transplantation. Bone Marrow Transplant. 2014;49(6):744–50. https://doi.org/10.1038/bmt.2014.55.
3. Dalle JH, Lucchini G, Balduzzi A, Ifversen M, Jahnukainen K, Macklon KT, Ahler A, Jarisch A, Ansari M, Beohou E, Bresters D, Corbacioglu S, Dalissier A, Diaz de Heredia Rubio C, Diesch T, Gibson B, Klingebiel T, Lankester A, Lawitschka A, Moffat R, Peters C, Poirot C, Saenger N, Sedlacek P, Trigoso E, Vettenranta K, Wachowiak J, Willasch A, von Wolff M, Yaniv I, Yesilipek A, Bader P. State-of-the-art fertility preservation in children and adolescents undergoing haematopoietic stem cell transplantation: a report on the expert meeting of the Paediatric Diseases Working Party (PDWP) of the European Society for Blood and Marrow Transplantation (EBMT) in Baden, Austria, 29-30 September 2015. Bone Marrow Transplant. 2017;52(7):1029–35. https://doi.org/10.1038/bmt.2017.21.
4. Balduzzi A, Dalle JH, Jahnukainen K, von Wolff M, Lucchini G, Ifversen M, Macklon KT, Poirot C, Diesch T, Jarisch A, Bresters D, Yaniv I, Gibson B, Willasch AM, Fadini R, Ferrari

L, Lawitschka A, Ahler A, Sänger N, Corbacioglu S, Ansari M, Moffat R, Dalissier A, Beohou E, Sedlacek P, Lankester A, De Heredia Rubio CD, Vettenranta K, Wachowiak J, Yesilipek A, Trigoso E, Klingebiel T, Peters C, Bader P. Fertility preservation issues in pediatric hematopoietic stem cell transplantation: practical approaches from the consensus of the pediatric diseases working party of the EBMT and the international BFM study group. Bone Marrow Transplant. 2017;52(10):1406–15. https://doi.org/10.1038/bmt.2017.147.

5. Borgmann-Staudt A, Rendtorff R, Reinmuth S, Hohmann C, Keil T, Schuster FR, Holter W, Ehlert K, Keslova P, Lawitschka A, Jarisch A, Strauss G. Fertility after allogeneic haematopoietic stem cell transplantation in childhood and adolescence. 2011; https://doi.org/10.1038/bmt.2011.78.
6. Zynda A, Reinmuth S, Pfitzer C, Hohmann C, Keil T, Borgmann-Staudt A. Childhood leukemia and its impact on graduation and having children: results from a national survey. Leuk Lymphoma. 2012;53(12):2419–22. https://doi.org/10.3109/10428194.2012.688965.
7. Matthews SJ, Picton H, Ernst E, Andersen CY. Successful pregnancy in a woman previously suffering from β-thalassemia following transplantation of ovarian tissue cryopreserved before puberty. Minerva Ginecol. 2018;70(4):432–5. https://doi.org/10.23736/S0026-4784.18.04240-5.
8. Dvorak CC, Gracia CR, Sanders JE, Cheng EY, Scott Baker K, Pulsipher MA, Petryk A. NCI, NHLBI/PBMTC first international conference on late effects after pediatric hematopoietic cell transplantation: endocrine challenges-thyroid dysfunction, growth impairment, bone health, & reproductive risks. Biol Blood Marrow Transplant. 2011;17:1725–38.
9. Chow EJ, Stratton KL, Leisenring WM, Oeffinger KC, Sklar CA, Donaldson SS, Ginsberg JP, Kenney LB, Levine JM, Robison LL, Shnorhavorian M, Stovall M, Armstrong GT, Green DM. (2016). Pregnancy after chemotherapy in male and female survivors of childhood cancer treated between 1970 and 1999: a report from the Childhood Cancer Survivor Study cohort. Lancet Oncol. 2016 May;17(5):567-576. oi: https://doi.org/10.1016/S1470-2045(16)00086-3.
10. Pfitzer C, Orawa H, Balcerek M, Langer T, Dirksen U, Keslova P, Zubarovskaya N, Schuster FR, Jarisch A, Strauss G, Borgmann-Staudt A. Dynamics of fertility impairment and recovery after allogeneic haematopoietic stem cell transplantation in childhood and adolescence: results from a longitudinal study. J Cancer Res Clin Oncol. 2015;141(1):135–42. https://doi.org/10.1007/s00432-014-1781-5.
11. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, Doney K, Storb R, Sullivan K, Witherspoon R, Appelbaum FR. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. Blood. 1996;87:3045–52.
12. Vatanen A, Wilhelmsson M, Borgström B, Gustafsson B, Taskinen M, Saarinen-Pihkala UM, Winiarski J, Jahnukainen K. Ovarian function after allogeneic hematopoietic stem cell transplantation in childhood and adolescence. Eur J Endocrinol. 2014;170(2):211–8. https://doi.org/10.1530/EJE-13-0694.
13. Assouline E, Crocchiolo R, Prebet T, Broussais F, Coso D, Gamerre M, Vey N, Blaise D, Courbiere B. Impact of reduced-intensity conditioning allogeneic stem cell transplantation on women's fertility. Clin Lymphoma Myeloma Leuk. 2013;13(6):704–10. https://doi.org/10.1016/j.clml.2013.05.014.
14. Panasiuk A, Nussey S, Veys P, Amrolia P, Rao K, Krawczuk-Rybak M, Leiper A. Gonadal function and fertility after stem cell transplantation in childhood: comparison of a reduced intensity conditioning regimen containing melphalan with a myeloablative regimen containing busulfan. Br J Haematol. 2015;170(5):719–26. https://doi.org/10.1111/bjh.13497.
15. Faraci M, Matthes-Martin S, Lanino E, Morreale G, Ferretti M, Giardino S, Micalizzi C, Balduzzi A. Two pregnancies shortly after transplantation with reduced intensity conditioning in chronic myeloid leukemia. Pediatr Transplant. 2016;20(1):158–61. https://doi.org/10.1111/petr.12630.
16. Yawn BP, Buchanan GR, Afenyi-Annan AN, Ballas SK, Hassell KL, James AH, Jordan L, Lanzkron SM, Lottenberg R, Savage WJ, Tanabe PJ, Ware RE, Murad MH, Goldsmith JC,

Ortiz E, Fulwood R, Horton A, John-Sowah J. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. JAMA. 2014;312(10):1033–48. https://doi.org/10.1001/jama.2014.10517.
17. Wang WC, Ware RE, Miller ST, Iyer RV, Casella JF, Minniti CP, Rana S, Thornburg CD, Rogers ZR, Kalpatthi RV, Barredo JC, Brown RC, Sarnaik SA, Howard TH, Wynn LW, Kutlar A, Armstrong FD, Files BA, Goldsmith JC, Waclawiw MA, Huang X, Thompson BW. BABY HUG investigators. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet. 2011;377(9778):1663–72. https://doi.org/10.1016/S0140-6736(11)60355-3.
18. McGann PT, Ware RE. Hydroxyurea therapy for sickle cell anemia. Expert Opin Drug Saf. 2015;14(11):1749–58. https://doi.org/10.1517/14740338.2015.1088827.
19. Elchuri SV, Williamson RS, Clark Brown R, Haight AE, Spencer JB, Buchanan I, Hassen-Schilling L, Brown MR, Mertens AC, Meacham LR. The effects of hydroxyurea and bone marrow transplant on anti-Müllerian hormone (AMH) levels in females with sickle cell anemia. Blood Cells Mol Dis. 2015;55(1):56–61. https://doi.org/10.1016/j.bcmd.2015.03.012.
20. Matthews M, Pollack R. (2017). Acute pain crisis in a patient with sickle cell disease undergoing ovarian simulation for fertility preservation prior to curative stem cell transplantation: case report and literature review. J Assist Reprod Genet. Nov;34(11):1445-1448.doi: https://doi.org/10.1007/s10815-017-1008-1.
21. Dovey S, Krishnamurti L, Sanfilippo J, Gunawardena S, Mclendon P, Campbell M, Alway S, Efymow B, Gracia C. Oocyte cryopreservation in a patient with sickle cell disease prior to hematopoietic stem cell transplantation: first report. J Assist Reprod Genet. 2012;29(3):265–9. https://doi.org/10.1007/s10815-011-9698-2.
22. Smith-Whitley K. Reproductive issues in sickle cell disease. Blood. 2014;124(24):3538–43. https://doi.org/10.1182/blood-2014-07-577619.
23. Schyrr F, Dolci M, Nydegger M, Canellini G, Andreu-Ullrich H, Joseph JM, Diezi M, Cachat F, Rizzi M, Renella R. Perioperative care of children with sickle cell disease: a systematic review and clinical recommendations. Am J Hematol. 2019;95(1):78–96. https://doi.org/10.1002/ajh.25626.
24. Fung EB, Harmatz PR, Lee PD, Milet M, Bellevue R, Jeng MR, Kalinyak KA, Hudes M, Bhatia S, Vichinsky EP, Multi-Centre Study of Iron Overload Research Group. Increased prevalence of iron-overload associated endocrinopathy in thalassaemia versus sickle-cell disease. Br J Haematol. 2006;135:574–82.
25. Carlberg KT, Singer ST, Vichinsky EP. Fertility and pregnancy in women with transfusion-dependent thalassemia. Hematol Oncol Clin North Am. 2018;32(2):297–315. https://doi.org/10.1016/j.hoc.2017.11.004.
26. Skordis N, Gourni M, Kanaris C, Toumba M, Kleanthous M, Karatzia N, Pavlides N, Angastiniotis M. The impact of iron overload and genotype on gonadal function in women with thalassaemia major. Pediatr Endocrinol Rev. 2004;2(Suppl 2):292–5.
27. Steward CG, Jarisch A. Haemopoietic stem cell transplantation for genetic disorders. Arch Dis Child. 2005;90:1259–63.

Severe Autoimmune Diseases

Melanie Henes, Michael von Wolff, and Joerg Henes

Indications and Prognosis

Autoimmune diseases often affect young women of reproductive age. Approximately 7% of all patients presenting at *Ferti*PROTEKT network clinics suffer from benign diseases, which include autoimmune diseases. Of these 7% of women, about 25% suffer from systemic lupus erythematosus (SLE) and 8% from vasculitis [1].

Above all, rheumatological systemic diseases such as connective tissue diseases and vasculitis and also haematological or neurological diseases such as multiple sclerosis, despite great therapeutic progress in recent years, continue to be an indication for the use of relatively undirected but highly immunosuppressive cytotoxic drugs. Cyclophosphamide (CYC) is used almost exclusively for this purpose orally or as intravenous pulse therapy. CYC also forms the cytotoxic central pillar for autologous stem cell transplantation, as the maximum therapy for immunosuppression in autoimmune diseases.

Diseases in which CYC therapy may be necessary:

M. Henes (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, Tuebingen, Germany
e-mail: melanie.henes@med.uni-tuebingen.de

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

J. Henes
Department of Internal Medicine II (Haematology, Oncology, Immunology and Rheumatology), University Hospital Tuebingen, Tuebingen, Germany
e-mail: joerg.henes@med.uni-tueningen.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_15

- Severe organ manifestations (glomerulonephritis, alveolitis or manifestations in the central nervous system) in *connective tissue diseases* (SLE, systemic sclerosis, Sjogren's syndrome, Sharp's syndrome, polymyositis or dermatomyositis)
- Severe organ manifestations (mostly pulmonary or renal) in anti-neutrophil cytoplasmic antibodies (*ANCA*) *associated vasculitis* (granulomatosis with polyangiitis [formerly: Wegener's granulomatosis], eosinophilic granulomatosis with polyangiitis [formerly: Churg–Strauss syndrome] or microscopic polyangiitis)
- Treatment-refractory forms of *large vascular vasculitis*, whereby only Takayasu arteritis occurs during reproductive age
- Autoimmune *neurological disease*s: e.g. multiple sclerosis
- *Non-malignant haematological diseases*: e.g. immune thrombocytopenia, acquired haemophilia, auto-immune haemolysis

With the exception of ANCA-associated vasculitides, these diseases usually peak before family planning is complete. A cure is not possible. However, by early diagnosis and initiation of the appropriate treatment, most patients can now be treated adequately on a permanent basis. As a result, their life expectancy has also become increasingly closer to that of the normal population, which means that the desire to have children and fertility preservation also plays an important role for these patients.

Gonadotoxity of the Treatments

The ovarian reserve, determined by the concentration of Anti Müllerian hormone (AMH), is limited in many autoimmune diseases due to the chronic disease per se and especially in cases of high disease activity [2–6]. For this reason, advice on fertility preservation should be given prior to CYC therapy, especially in autoimmune diseases.

CYC significantly increases the risk of premature ovarian insufficiency (POI) in autoimmune diseases. The percentages in the literature vary between 12 and 54% and are mainly influenced by the age of the patient at the time of therapy and the cumulative dose of CYC (Table 1).

The age and dose dependency of cyclophosphamide on ovarian toxicity are shown in a Chinese study of 216 women and in a study by Di Mario et al., in which ovarian toxicity was determined by AMH concentration [6, 16] (Fig. 1). According to these studies, other immunosuppressive drugs used in the treatment of SLE, such as mycophenolate, azathioprine, prednisolone, ciclosporin, tacrolimus and hydroxychloroquine, do not lead to a significant reduction in AMH concentration [6, 16].

Table 1 Studies on POI-rate after cyclophosphamide (CYC) treatment (SLE = systemic lupus erythematosus, GPA = granulomatosis with polyangiitis) and identified risk factor for premature ovarian insufficiency (POI)

Study	Origin of study	Diseases	Number of women	POI-rate (%)	Risk factors identified
Boumpas et al. 1993 [7]	USA	SLE	39	12–39	Age, CYC dose
Mc Dermott et al. 1996 [8]	UK	SLE	52	54	Age, CYC dose
Mok et al. 1998 [9]	China	SLE	70	26	Age, CYC dose
Ioannidis et al. 2002 [10]	Greece	SLE	67	31,3	Age, CYC dose disease duration
Huong et al. 2002 [11]	France	SLE, GPA	84	22,6	Age
Park et al. 2004 [12]	South Korea	SLE	67	14,9	Age
Singh et al. 2007 [13]	India	SLE	35	31,4	Cytochrome P450 polymorphism
Appenzeller et al. 2008 [14]	Canada	SLE	57 (CYC 750 mg/m^2) 50 (CYC 500 mg/m^2)	17,5 0	Age, CYC dose
Alarfaj et al. 2014 [15]	Saudi Arabia	SLE	188	13,1	Age, CYC dose
Di Mario et al. 2019 [6]	Italy	SLE	14	–	Age, CYC dose

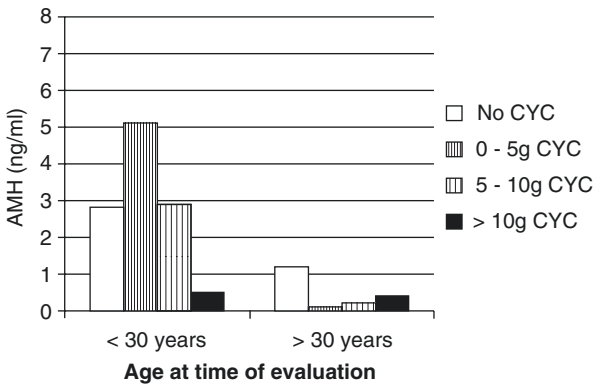


Fig. 1 AMH serum concentration after CYC treatment in women with SLE is dependent on age and dose (modified according to [16])

Probability of Exacerbation of the Underlying Disease

CYC treatment for autoimmune diseases is only indicated if there is high disease activity. A rapid initiation of therapy is usually necessary; however, the influence of fertility preservation therapy on the underlying disease must also be considered.

Due to the pathogenesis and gender distribution of many autoimmune diseases, it must be assumed that an increase in female hormones has a negative influence on the disease, and further exacerbation of the underlying disease can occur during ovarian stimulation for egg collection. Furthermore, other studies suggest that downregulation with a GnRH agonist has a positive effect on SLE [17]. A transfer of these findings to other autoimmune diseases is reasonable, but due to the rarity of the diseases, they have not been sufficiently and conclusively investigated.

Overall, there are only a few studies/recommendations on fertility preservation specifically for autoimmune diseases [18–21]. The other recommendations are mostly based on findings from the treatment of SLE patients. The European League against Rheumatism (EULAR) also includes fertility preservation in its 2017 recommendations [22].

Effectiveness and Risks of Fertility Preservation

The ovarian reserve is often reduced in autoimmune disease. Lawrenz et al. [3] and Di Mario et al. [6] found a 32% and 29% lower AMH concentration in women with systemic lupus erythematosus compared to a control collective. Lower AMH concentrations were also found in women with rheumatoid arthritis, Bechet's disease and spondyloarthritis [2], multiple sclerosis [5] and Takayasu's arteritis [4]. However, according to one study in lupus patients, AMH reduction appears to occur only in a severe form of autoimmune disease [6].

However, it is questionable whether the lowered AMH concentration also leads to fertility preservation measures being less effective. If oocytes are to be cryopreserved, the stimulation dose can often be adjusted. If ovarian tissue is cryopreserved, the AMH concentration plays a rather minor role. Important, however, is the density of primordial follicles, which in contrast to the AMH concentration, is not reduced in women with Hodgkin's lymphoma [23].

GnRH Agonists

The effectiveness of GnRH agonists (GnRHa) (see chapter "GnRH Agonists") has now been proven in patients with breast cancer (see chapter "Breast Cancer"). For autoimmune diseases only very limited data is available. However, it can be assumed that the data on efficacy in breast cancer can also be transferred to autoimmune diseases, since the risk of POI is comparable in both disease groups and the same cytotoxic drug is used (CYC).

Somers et al. [24] and Koga et al. [25] treated women with lupus erythematosus with CYC and GnRHa and compared the POI rate with a control group without GnRHa. The cumulative CYC doses administered were 12.9 g and approx. 5.0 g, respectively. The POI rate was 5% and 6% with GnRHa therapy and 30% and 50%

in the control group. Further studies investigated the effect of GnRHa [26] and its tolerability in children with SLE based on AMH concentration [27].

GnRHa can therefore be considered in individual cases as a singular method if a higher cumulative cyclophosphamide dose is planned.

Ovarian Stimulation

The procedure (see chapters "Ovarian Stimulation to Collect Oocytes" and "Cryopreservation of Unfertilized and Fertilized Oocytes") should be discussed individually if stimulation therapy for cryopreservation of fertilised or unfertilised eggs is to be carried out.

In principle, two risks should be emphasised:

1. Risk of Exacerbation of the Disease Under Stimulation

   In cases of connective tissue diseases in particular, especially SLE, stimulation can lead to a deterioration in disease activity. However, the available data are limited. Guballa et al. examined 17 women (10 with anti-phospholipid antibody syndrome (APS) and 7 with SLE) who underwent stimulation [28]. Stimulations with clomiphene citrate and with high-dose gonadotropins were included in the evaluation. No exacerbation was documented in women with APS. Women with SLE showed a slight exacerbation in 3/7 (43%) women in 3/16 (16%) stimulation cycles.
2. Risk of Thrombosis

   In general, the risk of thrombosis is increased in autoimmune disease, particularly in connective tissue diseases, and especially in SLE. Antiphospholipid antibodies are found in 40% of SLE patients, depending greatly on the patient's ethnicity [29–31]. The risk of thrombosis is highest in active APS and active SLE. According to a meta-analysis, if the serum marker "lupus anticoagulant" is increased, the risk of thrombosis increases by about six times, even in patients without SLE [32]. Other markers such as anticardiolipin antibodies, anti-β2 glycoprotein antibodies, anti-prothrombin antibodies, anti-phosphatidylserine antibodies and anti-phosphatidylethanolamine antibodies were only associated with a slight and insignificant increase in the risk of thrombosis in this study.

   There are little data available on the risk of thrombosis during stimulation. In the above-mentioned study by Guballa et al. [28], none of the 17 women stimulated with clomiphene citrate or gonadotropins had a thrombosis. However, all women received a thrombosis protection (heparin, aspirin or corticosteroids).

   In assisted reproduction, stimulation is also possible in SLE patients with special caution [28, 33]. In the event of an acute worsening of the underlying disease with the need for therapy escalation, the basic requirements for safe stimulation are not met. Therefore, this option should only be indicated with extreme caution in cases of active APS or SLE. Adequate thrombosis protection, depending on the risk profile, must be ensured [34].

Cryopreservation of Ovarian Tissue

Cryopreservation of ovarian tissue (see chapters "Removal of ovarian tissue" and "Transportation, cryopreservation and storage of ovarian tissue") is a good option for young women under the age of 35 and up to a maximum of approximately 40 years. Good pregnancy rates are particularly evident in women up to 35 years of age, and the method can also be successfully carried out in SLE patients [35, 36]. Since autoimmune diseases are chronic diseases, this method offers fertility preservation even if renewed CYC therapy is necessary. Due to the often reduced ovarian reserve, however, an adequate reserve should first be ensured by AMH measurement and determination of the AFC using ultrasound. A case report of a successful pregnancy in a patient with SLE after retransplantation of cryopreserved ovarian tissue is available [36].

Practical Approach

The choice of fertility preservation methods is always an individual decision, which should be made in close consultation with the patient, the gynaecologists and rheumatologists in charge.

In principle, patients should be introduced to a reproductive medicine centre as early as possible in order to ensure the greatest possible time frame for the implementation of fertility preservation methods. Figure 2 shows the procedure for carrying out fertility preservation measures for autoimmune diseases.

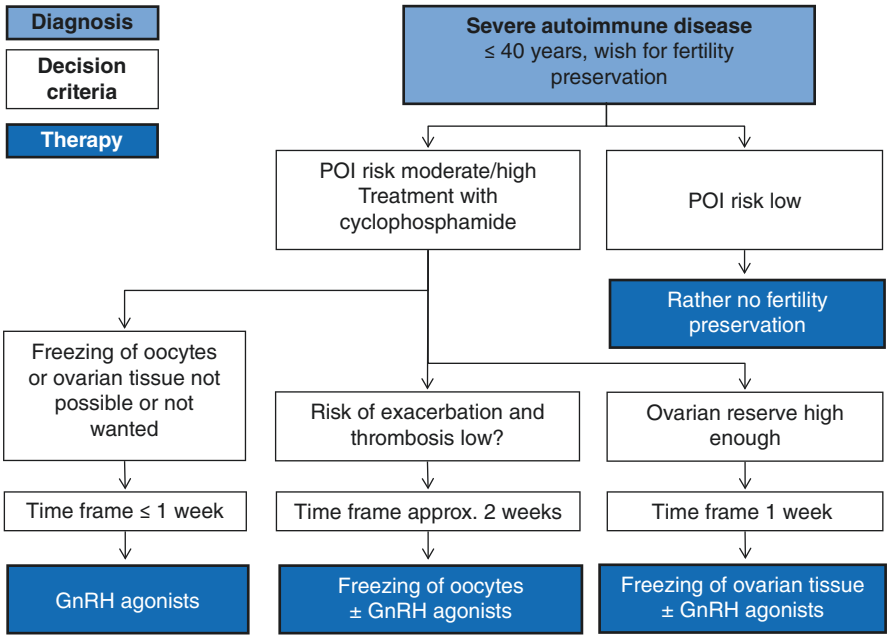


Fig. 2 Algorithm for fertility preservation in women with autoimmune diseases

References

1. von Wolff M, Dittrich R, Liebenthron J, Nawroth F, Schuring AN, Bruckner T, et al. Fertility-preservation counselling and treatment for medical reasons: data from a multinational network of over 5000 women. Reprod Biomed Online. 2015;31(5):605–12.
2. Henes M, Froeschlin J, Taran FA, Brucker S, Rall KK, Xenitidis T, et al. Ovarian reserve alterations in premenopausal women with chronic inflammatory rheumatic diseases: impact of rheumatoid arthritis, Behcet's disease and spondyloarthritis on anti-Mullerian hormone levels. Rheumatology (Oxford). 2015;54(9):1709–12.
3. Lawrenz B, Henes J, Henes M, Neunhoeffer E, Schmalzing M, Fehm T, et al. Impact of systemic lupus erythematosus on ovarian reserve in premenopausal women: evaluation by using anti-Mullerian hormone. Lupus. 2011;20(11):1193–7.
4. Mont'Alverne AR, Pereira RM, Yamakami LY, Viana VS, Baracat EC, Bonfa E, et al. Reduced ovarian reserve in patients with Takayasu arteritis. J Rheumatol. 2014;41(10):2055–9.
5. Thone J, Kollar S, Nousome D, Ellrichmann G, Kleiter I, Gold R, et al. Serum anti-Mullerian hormone levels in reproductive-age women with relapsing-remitting multiple sclerosis. Mult Scler. 2015;21(1):41–7.
6. Di Mario C, Petricca L, Gigante MR, Barini A, Barini A, Varriano V, et al. Anti-Mullerian hormone serum levels in systemic lupus erythematosus patients: influence of the disease severity and therapy on the ovarian reserve. Endocrine. 2019;63(2):369–75.
7. Boumpas DT, Austin HA 3rd, Vaughan EM, Yarboro CH, Klippel JH, Balow JE. Risk for sustained amenorrhea in patients with systemic lupus erythematosus receiving intermittent pulse cyclophosphamide therapy. Ann Intern Med. 1993;119(5):366–9.
8. McDermott EM, Powell RJ. Incidence of ovarian failure in systemic lupus erythematosus after treatment with pulse cyclophosphamide. Ann Rheum Dis. 1996;55(4):224–9.

9. Mok CC, Lau CS, Wong RW. Risk factors for ovarian failure in patients with systemic lupus erythematosus receiving cyclophosphamide therapy. Arthritis Rheum. 1998;41(5):831–7.
10. Ioannidis JP, Katsifis GE, Tzioufas AG, Moutsopoulos HM. Predictors of sustained amenorrhea from pulsed intravenous cyclophosphamide in premenopausal women with systemic lupus erythematosus. J Rheumatol. 2002;29(10):2129–35.
11. Huong DL, Amoura Z, Duhaut P, Sbai A, Costedoat N, Wechsler B, et al. Risk of ovarian failure and fertility after intravenous cyclophosphamide. A study in 84 patients. J Rheumatol. 2002;29(12):2571–6.
12. Park MC, Park YB, Jung SY, Chung IH, Choi KH, Lee SK. Risk of ovarian failure and pregnancy outcome in patients with lupus nephritis treated with intravenous cyclophosphamide pulse therapy. Lupus. 2004;13(8):569–74.
13. Singh G, Saxena N, Aggarwal A, Misra R. Cytochrome P450 polymorphism as a predictor of ovarian toxicity to pulse cyclophosphamide in systemic lupus erythematosus. J Rheumatol. 2007;34(4):731–3.
14. Appenzeller S, Blatyta PF, Costallat LT. Ovarian failure in SLE patients using pulse cyclophosphamide: comparison of different regimes. Rheumatol Int. 2008;28(6):567–71.
15. Alarfaj AS, Khalil N. Fertility, ovarian failure, and pregnancy outcome in SLE patients treated with intravenous cyclophosphamide in Saudi Arabia. Clin Rheumatol. 2014;33(12):1731–6.
16. Mok CC, Chan PT, To CH. Anti-Mullerian hormone and ovarian reserve in systemic lupus erythematosus. Arthritis Rheum. 2013;65(1):206–10.
17. Mok CC, Wong RW, Lau CS. Ovarian failure and flares of systemic lupus erythematosus. Arthritis Rheum. 1999;42(6):1274–80.
18. Henes M, Henes JC, Neunhoeffer E, Von Wolff M, Schmalzing M, Kotter I, et al. Fertility preservation methods in young women with systemic lupus erythematosus prior to cytotoxic therapy: experiences from the FertiPROTEKT network. Lupus. 2012;21(9):953–8.
19. Henes JC, Henes M, von Wolff M, Schmalzing M, Kotter I, Lawrenz B. Fertility preservation in women with vasculitis: experiences from the FertiPROTEKT network. Clin Exp Rheumatol. 2012;30(1 Suppl 70):S53–6.
20. Condorelli M, Demeestere I. Challenges of fertility preservation in non-oncological diseases. Acta Obstet Gynecol Scand. 2019;98(5):638–6.
21. Elizur SE, Chian RC, Pineau CA, Son WY, Holzer HE, Huang JY, et al. Fertility preservation treatment for young women with autoimmune diseases facing treatment with gonadotoxic agents. Rheumatology (Oxford). 2008;47(10):1506–9.
22. Andreoli L, Bertsias GK, Agmon-Levin N, Brown S, Cervera R, Costedoat-Chalumeau N, et al. EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome. Ann Rheum Dis. 2017;76(3):476–85.
23. Liebenthron JRJ, van der Ven H, Saenger N, Kruessel JS, von Wolff M. Serum anti-Mullerian hormone concentration and follicle density throughout reproductive life and in different diseases - implications in fertility preservation. Hum Reprod. 2019;34(12):2513–22.
24. Somers EC, Marder W, Christman GM, Ognenovski V, McCune WJ. Use of a gonadotropin-releasing hormone analog for protection against premature ovarian failure during cyclophosphamide therapy in women with severe lupus. Arthritis Rheum. 2005;52(9):2761–7.
25. Koga T, Umeda M, Endo Y, Ishida M, Fujita Y, Tsuji S, et al. Effect of a gonadotropin-releasing hormone analog for ovarian function preservation after intravenous cyclophosphamide therapy in systemic lupus erythematosus patients: a retrospective inception cohort study. Int J Rheum Dis. 2018;21(6):1287–92.
26. Marder W, McCune WJ, Wang L, Wing JJ, Fisseha S, McConnell DS, et al. Adjunctive GnRH-a treatment attenuates depletion of ovarian reserve associated with cyclophosphamide therapy in premenopausal SLE patients. Gynecol Endocrinol. 2012;28(8):624–7.
27. Brunner HI, Silva CA, Reiff A, Higgins GC, Imundo L, Williams CB, et al. Randomized, double-blind, dose-escalation trial of triptorelin for ovary protection in childhood-onset systemic lupus erythematosus. Arthritis Rheumatol. 2015;67(5):1377–85.

28. Guballa N, Sammaritano L, Schwartzman S, Buyon J, Lockshin MD. Ovulation induction and in vitro fertilization in systemic lupus erythematosus and antiphospholipid syndrome. Arthritis Rheum. 2000;43(3):550–6.
29. Chung WS, Lin CL, Chang SN, Lu CC, Kao CH. Systemic lupus erythematosus increases the risks of deep vein thrombosis and pulmonary embolism: a nationwide cohort study. J Thromb Haemost. 2014;12(4):452–8.
30. Mok CC, Tang SS, To CH, Petri M. Incidence and risk factors of thromboembolism in systemic lupus erythematosus: a comparison of three ethnic groups. Arthritis Rheum. 2005;52(9):2774–82.
31. Bazzan M, Vaccarino A, Marletto F. Systemic lupus erythematosus and thrombosis. Thromb J. 2015;13:16.
32. Reynaud Q, Lega JC, Mismetti P, Chapelle C, Wahl D, Cathebras P, et al. Risk of venous and arterial thrombosis according to type of antiphospholipid antibodies in adults without systemic lupus erythematosus: a systematic review and meta-analysis. Autoimmun Rev. 2014;13(6):595–608.
33. Ragab A, Barakat R, Ragheb M, State O, Badawy A. Subfertility treatment in women with systemic lupus erythematosus. J Obstet Gynaecol. 2012;32(6):569–71.
34. Ostensen M, Andreoli L, Brucato A, Cetin I, Chambers C, Clowse ME, et al. State of the art: reproduction and pregnancy in rheumatic diseases. Autoimmun Rev. 2015;14(5):376–86.
35. Van der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krussel J, et al. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. Hum Reprod. 2016;31(9):2031–41.
36. Chehab G, Krussel J, Fehm T, Fischer-Betz R, Schneider M, Germeyer A, et al. Successful conception in a 34-year-old lupus patient following spontaneous pregnancy after auto-transplantation of cryopreserved ovarian tissue. Lupus. 2019;28(5):675–80. https://doi.org/10.1177/0961203319839482.

Endometriosis

Alexandra S. Kohl Schwartz, Sara Imboden, and Michael von Wolff

Introduction

Ten percent of women of fertile age are affected by endometriosis, and 30–50% of these women suffer from infertility [1, 2] Due to the chronic progression of the disease, there is a risk of a reduction in the ovarian reserve in these women, both because of the pathophysiology of the disease and because of the possible iatrogenic injury to the ovaries by surgical intervention. Approximately 40–50% of young women experience a recurrence of endometriosis before trying to become pregnant [3, 4].

Fertility preservation in endometriosis follows different principles than in malignant diseases, where there is only a short window of time available before gonadotoxic treatment is started.

Fertility preservation includes carefully determining the indications for a restorative and at the same time fertility-preserving surgical intervention. The aim should also be to try for a pregnancy as early as possible. Fertility preservation measures in the sense of ovarian stimulation and cryopreservation of oocytes (see chapters "Ovarian Stimulation to Collect Oocytes" and "Cryopreservation of Unfertilized and Fertilized Oocytes") are only relevant in young women who are at risk of a relevant reduction in fertility because of disease progression and who do not yet have a current desire to have children or are not yet able to realise it.

A. S. Kohl Schwartz (✉) · M. von Wolff
Division of Gynecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: alexandra.kohl-schwartz@insel.ch; Michael.vonWolff@insel.ch

S. Imboden
Department of Obstetrics and Gynecology, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Sara.Imboden@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_16

Classification of Endometriosis

rASRM Classification

Endometriosis is divided into several stages, depending on its spread. One of the best-known classifications is the rASRM classification of the American Society for Reproductive Medicine (ASRM) (Fig. 1). Four stages are determined using a scoring system. The resulting rASRM score shows only a weak correlation with symptoms such as dysmenorrhoea and dyspareunia and with the degree and risk of infertility [5, 6].

According to the rASRM classification, endometriosis is divided into the following four stages:

- Stage I (minimal): 1–5 points
- Stage II (mild): 6–15 points
- Stage III (moderate): 16–40 points
- Stage IV (severe): >40 points

ENZIAN Classification

The ENZIAN classification was developed, because the rASRM classification lacks the description of retroperitoneal structures in deeply infiltrating endometriosis. Deep-infiltrating endometriosis is often associated with infertility due to the restricted mobility of the pelvic organs.

The ENZIAN classification should be seen as a complementary classification [8]. Analogous to an oncological TNM (tumour, node = lymph nodes, metastases) classification, it describes the endometriosis lesions in three different anatomical compartments and spatial axes and assigns a severity level to each of them.

- Compartment A comprises the rectovaginal space, extending from the rectouterine pouch towards the vagina.
- Compartment B describes the space lateral to the uterosacral ligaments, extending towards the pelvic wall.
- Compartment C describes the area from the rectovaginal space towards the rectum and includes the rectum.

Pathophysiology of Infertility in Endometriosis

In moderate to severe stages of endometriosis anatomy is altered. The tubal uptake of the oocyte can be severly disturbed due to limited mobility or the closure of the tube(s).

AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE
REVISED CLASSIFICATION OF ENDOMETRIOSIS

Patient's Name _____ Date_____

Stage I (Minimal): 1-5
Stage II (Mild): 6-15
Stage III (Moderate): 16-40
Stage IV (Severe): >40
Total: _____

Laparoscopy_____ Laparotomy_____ Photography__
Laparoscopic Treatment_____
_____
Prognosis_____

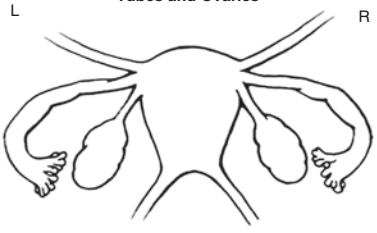
PERITONEUM	ENDOMETRIOSIS	< 1cm	1-3 cm	>3 cm
	Superficial	1	2	4
	Deep	2	4	6
OVARY	R Superficial	1	2	4
	Deep	4	16	20
	L Superficial	1	2	4
	Deep	4	16	20
	POSTERIOR CULDESAC OBLITERATION	Partial	Complete	
		4	40	
OVARY	ADHESIONS	< 1/3 Enclosure	1/3-2/3 Enclosure	>2/3 Enclosure
	R Filmy	1	2	4
	Dense	4	8	16
	L Filmy	1	2	4
	Dense	4	8	16
TUBES	R Filmy	1	2	4
	Dense	4•	8*	16
	L Filmy	1	2	4
	Dense	4•	8*	16

*If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16.

Additional Endometriosis:_____
_____
_____
_____

Associated Pathology:_____
_____
_____
_____

To Be Used with Normal Tubes and Ovaries



To Be Used with Abnormal Tubes and Ovaries

Fig. 1 rASRM classifications of endometriosis, describing the spread of endometriosis [7]

The endometriosis foci lead to an inflammatory reaction, which causes various fertility-relevant systems to dysfunction. Higher concentrations of inflammatory cytokines can be detected in the peritoneal fluid [9] as well as in the follicular fluid [10].

The peritoneal fluid which has been altered by inflammation probably influences sperm [11] and tube motility [12].

The endometrium is negatively influenced by free radicals in the peritoneal fluid [13–15]. In women with endometriosis, it contains increased concentrations of pro-inflammatory cytokines [16] and exhibits dysregulation of the progesterone receptor, which can lead to progesterone resistance [17] and thus to a reduced effect of progesterone on the endometrium. This may result in luteal phase dysfunction [18–20].

Ovarian function is influenced by the mechanical stretching of the ovarian cortex in the peripheral area of the endometrioma. Cystic fluid from the endometrioma can lead to an increased concentration of iron in the follicles [21, 22].

As a whole, these factors have a negative effect on conception and embryonic development [12], which is also reflected in a higher miscarriage rate in women with endometriosis [23].

Fertility Preservation Measures

Spontaneous Pregnancy

In patients with endometriosis, the primary goal should be pregnancy if possible, either spontaneously or – if necessary – with the help of reproductive medicine. The chances of a spontaneous pregnancy can be estimated using the Endometriosis Fertility Index (EFI) [31] (Fig. 2). This scoring system calculates the chance of pregnancy based on the functionality of the tubes and ovaries, depending on the age of the woman and the duration of infertility (Fig. 3). The effectiveness of this score has been confirmed by other groups [32].

Surgical Techniques

Surgical clearance not only reduces symptoms, such as dysmenorrhoea and dyspareunia, but can also enhance fertility [33].

However, surgery can reduce the ovarian reserve by damaging the ovary itself and the surrounding tissue [28, 34]. A meta-analysis in 2015 showed that after surgery to remove endometrioma on the affected ovary, fewer oocytes were obtained with IVF and more stimulation cycles were discontinued (Table 1) [30].

ENDOMETRIOSIS FERTILITY INDEX (EFI)
SURGERY FORM

LEAST FUNCTION (LF) SCORE AT CONCLUSION OF SURGERY

Score	Description		Left	Right
4	= Normal	Fallopian Tube		
3	= Mild Dysfunction			
2	= Moderate Dysfunction	Fimbria		
1	= Severe Dysfunction			
0	= Absent or Nonfunctional	Ovary		

To calculate the LF score, add together the lowest score for the left side and the lowest score for the right side. If an ovary is absent on one side, the LF score is obtained by doubling the lowest score on the side with the ovary.

Lowest Score [ ] (Left) + [ ] (Right) = [ ] (LF Score)

ENDOMETRIOSIS FERTILITY INDEX (EFI)

Historical Factors			Surgical Factors		
Factor	**Description**	**Points**	**Factor**	**Description**	**Points**
Age			LF Score		
	If age is ≤ 35 years	2		If LF Score = 7 to 8 (high score)	3
	If age is 36 to 39 years	1		If LF Score = 4 to 6 (moderate score)	2
	If age is ≥ 40 years	0		If LF Score = 1 to 3 (low score)	0
Years Infertile			AFS Endometriosis Score		
	If years infertile is ≤ 3	2		If AFS Endometriosis Lesion Score is < 16	1
	If years infertile is > 3	0		If AFS Endometriosis Lesion Score is ≥ 16	0
Prior Pregnancy			AFS Total Score		
	If there is a history of a prior pregnancy	1		If AFS total score is < 71	1
	If there is no history of prior pregnancy	0		If AFS total score is ≥ 71	0
Total Historical Factors			**Total Surgical Factors**		

EFI = TOTAL HISTORICAL FACTORS + TOTAL SURGICAL FACTORS: [ ] (Historical) + [ ] (Surgical) = [ ] **EFI Score**

Fig. 2 Endometriosis Fertility Index (EFI), which describes the chances of spontaneous conception in patients with endometriosis [31]

On the other hand, endometriomas per se cause a reduction in the ovarian reserve (Table 1) [21, 22], and under certain conditions, it is advisable to seek surgical removal. It has been suggested by individual authors and in the ESHRE guideline [33] that a cystectomy should be performed with an endometrioma size of ≥3 cm, also to rule out malignancy. However, in most cases, it is advisable to determine the indication for surgery individually. The expected surgical damage (Table 1) (reduction in the ovarian reserve) plays a role as well, as does the potential benefit (better accessibility of the ovaries and follicles during follicle aspiration). The patient's current pain situation must also be considered when deciding upon the indication for surgery. Dyspareunia per se can greatly reduce the chances of pregnancy.

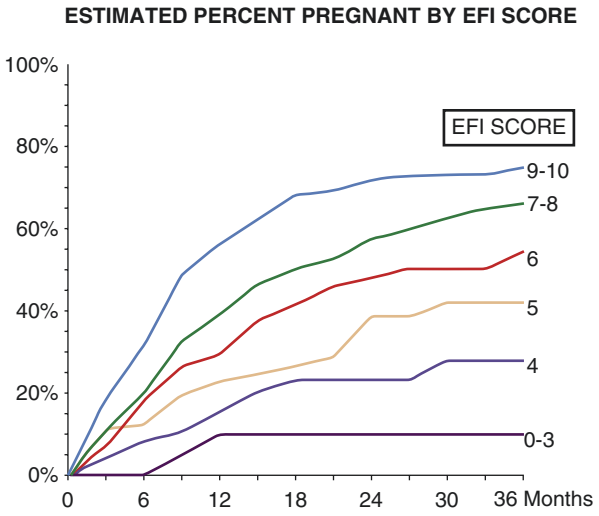


Fig. 3 Endometriosis Fertility Index (EFI), presentation of the estimated conception chances depending on the EFI score [31]

Table 1 Effects of ovarian endometriosis on conception

	Effect on ovarian function	Effect on ovarian reserve	Effect on IVF treatment	Effect on pregnancy rate
Ovarian endometriomas	Mechanical stretching of the ovarian cortex by endometrioma leads to increased ovarian fibrosis and reduced follicle density in 55% of cases [22], and higher iron content in the follicles [21].	Reduction of the ovarian reserve through bilateral endometrioma: Lower serum AMH concentration with bilateral endometrioma: 1.3 ng/mL (Median, interquartile range: 0.5–2.5) versus 2.0 ng/mL (1.1–3.6) with unilateral endometrioma [24].	For bilateral endometrioma, aspiration of fewer oocytes, but the same pregnancy rate as for unilateral endometrioma [25, 26].	Ovulation occurs less frequently in ovaries with endometrioma (31%; 95% CI: 22–43%) [27].
Endometriosis related ovarian surgery		Reduction in serum AMH concentration. Meta-analysis: decrease in AMH concentration by 1.5 ng/mL (95% CI 1.04–2) [28]. Significantly higher decrease after bilateral resection [29].	Higher risk of poor response (<4 oocytes) OR = 2.1; 95% CI: 1.1–4. [25, 26]. Lower number of oocytes retrieved; Mean difference: -2.37 (−3.55 to −1.70) [30].	Reduced pregnancy rate with IVF treatment versus healthy controls (OR 0.53 (95% CI: 0.33–0.84) [30].

The surgical technique has an influence on tissue damage. To reduce the risk of recurrence and alleviate pain symptoms, the cyst should be carefully enucleated. If this is not possible, laser vaporisation is an alternative, especially in cases of bilateral involvement or ovaries which have previously been operated on. Bipolar coagulation and ovariectomy should be avoided [35, 36]. The operation should always be minimally invasive to prevent adhesions. This requires particular surgical expertise.

Cryopreservation of Oocytes

The indication for fertility preservation is given if the bilateral damage to the ovaries is foreseeable, if the woman wants or has to postpone her desire for children and still has a sufficient ovarian reserve.

As a rule, only ovarian stimulation and cryopreservation of mature oocytes are pertinent as a fertility preservation measure. The cryopreservation of ovarian tissue is usually not relevant in endometriosis patients, since tissue removal would further reduce the ovarian reserve and the spontaneous chance of pregnancy after transplantation of ovarian tissue is likely to be low due to intra-abdominal adhesions.

Data on the cryopreservation of oocytes as a fertility-protective measure in women with endometriosis are limited. A case study [37] was published on the cryopreservation of 21 oocytes which have not yet been thawed. A recently published case series showed data from 49 endometriosis patients and a total of 70 cryopreservation cycles. The cohort [38] was divided into three subgroups according to their endometriosis phenotype: peritoneal, ovarian and deep infiltrating. The mean number of cryopreserved oocytes per cycle was 7.2 ± 4.9 in all women. After surgical treatment of ovarian lesions, significantly fewer cells (on average 5.3 ± 3.7 oocytes) could be cryopreserved.

Based on the data from egg donation therapies, problem-free cryopreservation and storage of the oocytes over several years can be assumed. The chances of success depend greatly on the number of oocytes collected and the age of the woman at removal, as well as on the expertise of the centre with cryopreservation [39]. Data on the success rate depending on the age and the number of cryopreserved oocytes for women without endometriosis can be found in chapter “Cryopreservation of Unfertilized and Fertilized Oocytes”. It should be noted, however, that the chances of success are likely to be lower in case of endometriosis due to the factors mentioned above.

Ovarian stimulation to create a fertility reserve only makes sense if the associated chance of a later pregnancy is sufficiently high. The women should therefore be relatively young and still have a sufficient ovarian reserve. The age limit of 35 years and an AMH $\geq$ 1 ng/mL mentioned in Fig. 4 are not based on scientific studies but follows clinical experience.

When determining the indication for cryopreservation of oocytes, the risks of this measure must be considered. Seyhan et al. [40] showed that the volume of endometriomas increased significantly from approx. 22–25 mL and thus by approx. 10% in approx. 80% of cases during a stimulation cycle. The increase in size was

more pronounced in large endometriomas. Assuming that this increase in size is due to high estrogen concentrations, letrozole could be used in addition to gonadotrophins (see chapter "Ovarian Stimulation to Collect Oocytes"). Kim et al. [41] showed that the estrogen concentration was about two-third lower with additional treatment with letrozole, but the oocyte count did not differ. However, it is unclear whether letrozole can also prevent the growth of endometriomas under stimulation.

It should also be noted that follicle aspiration is associated with an increased risk of bleeding and infection due to the often-altered anatomical conditions and endometriomas [42–44]. Endometriomas should not be punctured and antibiotic therapy (e.g. cefuroxime 1.5 mg 3x/day i.v. or 500mg 2x/day orally for up to 4 days) should be considered in the event of an accidental aspiration [44].

Practical Approach

The first step is to decide on the priorities of the procedure. Should the primary goal be spontaneous conception or a surgical intervention? Is the aim a fertility preservation measure with cryopreservation of oocytes? It is also possible that a surgical intervention is planned primarily, but cryopreservation of oocytes is carried out beforehand, as the risk of surgically induced reduction of the ovarian reserve is high.

Ovarian stimulation is performed in the same way as in classical IVF. Drug treatment for endometriosis (e.g. with 2 mg dienogest per day) can probably be continued until 2–3 days before the start of stimulation, as dienogest has a half-life of only 9 h [45]. Continuation of progestin administration during stimulation is not recommended, since otherwise, as with luteal phase stimulation (see chapter "Ovarian Stimulation to Collect Oocytes"), fewer oocytes could be obtained.

The data regarding the benefit of a long or ultra-long agonist protocol with several weeks of prior downregulation is not clear [46]. Although some studies have described a higher birth rate with longer downregulation, this does not mean that this is also beneficial for the cryopreservation of oocytes. It is suspected that long downregulation leads to a beneficial reduction in the endometriosis-related inflammatory reaction, but also to atrophy of adenomyosis, which is often associated with higher grade endometriosis [47]. Both are beneficial for implantation and early embryonic development. However, this positive effect is not relevant in the cryopreservation of oocytes, as no transfer takes place.

For ovarian stimulation the antagonist protocol in combination with ovulation induction with GnRH agonists should be used (see chapter "Ovarian Stimulation to Collect Oocytes"). This protocol reduces the risk of ovarian hyperstimulation syndrome. Premature luteolysis with the associated drop in estrogen concentration should also be beneficial. Surgical treatment of endometriosis should be performed 2 weeks after follicular aspiration at the earliest, i.e. after the end of the luteal phase, as this is when the estrogen and progesterone concentrations have fallen and the corpora lutea have degenerated, which should lead to a lower risk of bleeding.

It is unclear how many stimulation cycles should be carried out and whether they should follow one another directly or if there should be an endocrine therapy phase in between. These questions must be clarified individually, depending on the number of oocytes obtained, the pain caused by endometriosis and the patient's wishes. If the oocytes are later thawed, fertilised and an embryo transfer is carried out, luteal phase support is mandatory, as luteal phase dysfunction is suspected in severe endometriosis [18–20] (Figs. 4 and 5).

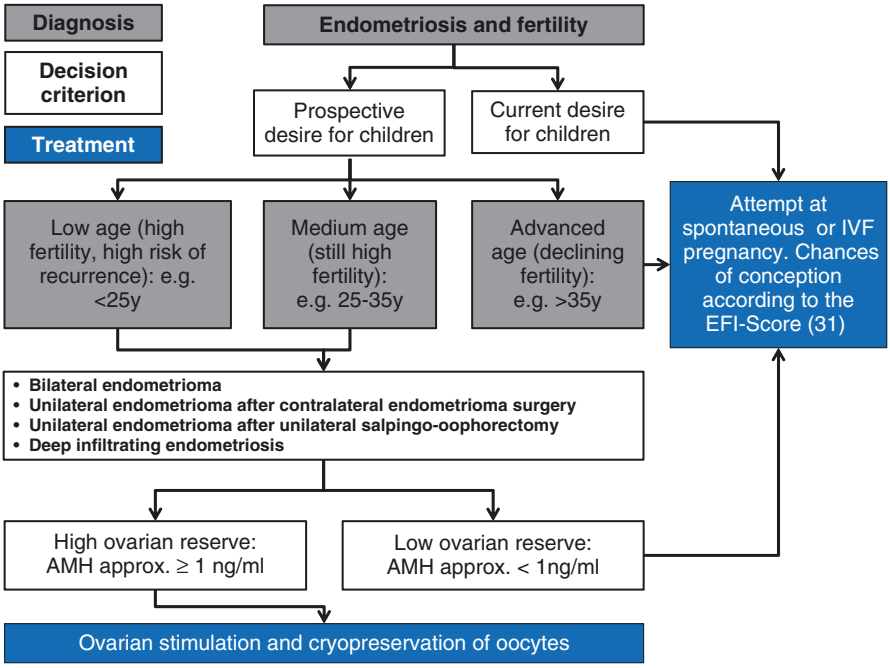


Fig. 4 Algorithm for fertility preservation in patients with endometriosis

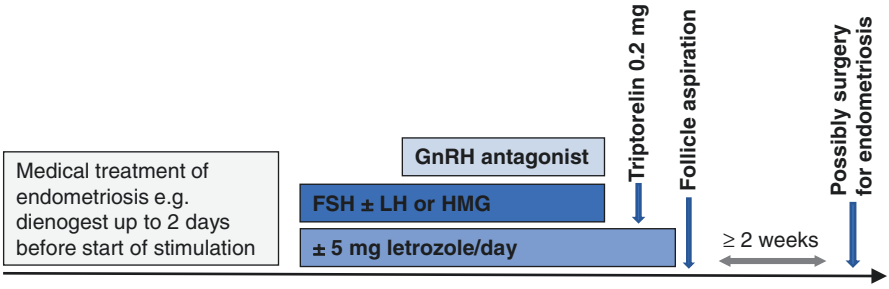


Fig. 5 Stimulation protocol for the cryopreservation of oocytes in patients with endometriosis

References

1. Viganò P, Parazzini F, Somigliana E, Vercellini P. Endometriosis: epidemiology and aetiological factors. Best Pract Res Clin Obstet Gynaecol. 2004;18:177–200.
2. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. Lancet. 2010;376:730–8.
3. Brosens I, Gordts S, Benagiano G. Endometriosis in adolescents is a hidden, progressive and severe disease that deserves attention, not just compassion. Hum Reprod. 2013;28:2026–31.
4. Benagiano G, Guo S-W, Puttemans P, Gordts S, Brosens I. Progress in the diagnosis and management of adolescent endometriosis: an opinion. Reprod Biomed Online. 2018;36:102–14.
5. Vercellini P, Trespidi L, De Giorgi O, Cortesi I, Parazzini F, Crosignani PG. Endometriosis and pelvic pain: relation to disease stage and localization. Fertil Steril. 1996;65:299–304.
6. Guzick DS, Silliman NP, Adamson GD, Buttram VC, Canis M, Malinak LR, Schenken R. Prediction of pregnancy in infertile women based on the American Society for Reproductive Medicine's revised classification of endometriosis. Fertil Steril. 1997;67:822–9.
7. American Society for Reproductive. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997;67:817–21.
8. Haas D, Chvatal R, Habelsberger A, Wurm P, Schimetta W, Oppelt P. Comparison of revised American Fertility Society and ENZIAN staging: a critical evaluation of classifications of endometriosis on the basis of our patient population. Fertil Steril. 2011;95:1574–8.
9. Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. Fertil Steril. 2001;75:1–10.
10. Wu G, Bersinger NA, Mueller MD, von Wolff M. Intrafollicular inflammatory cytokines but not steroid hormone concentrations are increased in naturally matured follicles of women with proven endometriosis. J Assist Reprod Genet. 2017;34:357–64.
11. Oral E, Seli E, Bahtiyar MO, Olive DL, Arici A. Growth-regulated alpha expression in the peritoneal environment with endometriosis. Obstet Gynecol. 1996;88:1050–6.
12. Lyons RA, Djahanbakhch O, Saridogan E, Naftalin AA, Mahmood T, Weekes A, Chenoy R. Peritoneal fluid, endometriosis, and ciliary beat frequency in the human fallopian tube. Lancet. 2002;360:1221–2.
13. Ota H, Igarashi S, Sato N, Tanaka H, Tanaka T. Involvement of catalase in the endometrium of patients with endometriosis and adenomyosis. Fertil Steril. 2002;78:804–9.
14. Grandi G, Mueller MD, Bersinger NA, Facchinetti F, McKinnon BD. The association between progestins, nuclear receptors expression and inflammation in endometrial stromal cells from women with endometriosis. Gynecol Endocrinol. 2017;33:712–5.
15. McKinnon BD, Kocbek V, Nirgianakis K, Bersinger NA, Mueller MD. Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics. Hum Reprod Update. 2016;22:382–403.
16. Patel BG, Rudnicki M, Yu J, Shu Y, Taylor RN. Progesterone resistance in endometriosis: origins, consequences and interventions. Acta Obstet Gynecol Scand. 2017;96:623–32.
17. McKinnon B, Mueller M, Montgomery G. Progesterone resistance in endometriosis: an acquired property? Trends Endocrinol Metab. 2018;29:535–48.
18. Giudice LC. Endometriosis. N Engl J Med. 2010;362:2389–98.
19. Santamaria X, Massasa EE, Taylor HS. Migration of cells from experimental endometriosis to the uterine endometrium. Endocrinology. 2012;153:5566–74.
20. Vallvé-Juanico J, Houshdaran S, Giudice LC. The endometrial immune environment of women with endometriosis. Hum Reprod Update. 2019;25:565–92.
21. Sanchez AM, Papaleo E, Corti L, Santambrogio P, Levi S, Vigano P, Candiani M, Panina-Bordignon P. Iron availability is increased in individual human ovarian follicles in close proximity to an endometrioma compared with distal ones. Hum Reprod. 2014;29:577–83.
22. Kitajima M, Defrère S, Dolmans M-M, Colette S, Squifflet J, Van Langendonckt A, Donnez J. Endometriomas as a possible cause of reduced ovarian reserve in women with endometriosis. Fertil Steril. 2011;96:685–91.

23. Kohl Schwartz AS, Wölfler MM, Mitter V, Rauchfuss M, Haeberlin F, Eberhard M, von Orelli S, Imthurn B, Imesch P, Fink D, Leeners B. Endometriosis, especially mild disease: a risk factor for miscarriages. Fertil Steril. 2017;108:806–14.
24. Somigliana E, Marchese MA, Frattaruolo MP, Berlanda N, Fedele L, Vercellini P. Serum anti-mullerian hormone in reproductive aged women with benign ovarian cysts. Eur J Obstet Gynecol Reprod Biol. 2014;180:142–7.
25. Bourdon M, Raad J, Dahan Y, Marcellin L, Maignien C, Even M, Pocate-Cheriet K, Lamau M, Santulli P, Chapron C. Endometriosis and ART: A prior history of surgery for OMA is associated with a poor ovarian response to hyperstimulation. PLoS One. 2018;13:e0202399.
26. Benaglia L, Bermejo A, Somigliana E, Faulisi S, Ragni G, Fedele L, Garcia-Velasco J. In vitro fertilization outcome in women with unoperated bioateral endometriomas. Fertil Steril. 2013;99:1714–9.
27. Benaglia L, Somigliana E, Vercellini P, Abbiati A, Ragni G, Fedele L. Endometriotic ovarian cysts negatively affect the rate of spontaneous ovulation. Hum Reprod. 2009;24:2183–6.
28. Raffi F, Metwally M, Amer S. The impact of excision of ovarian endometrioma on ovarian reserve: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2012;97:3146–54.
29. Hirokawa W, Iwase A, Goto M, Takikawa S, Nagatomo Y, Nakahara T, Bayasula B, Nakamura T, Manabe S, Kikkawa F. The post-operative decline in serum anti-Müllerian hormone correlates with the bilaterality and severity of endometriosis. Hum Reprod. 2011;26:904–10.
30. Hamdan M, Dunselman G, Li TC, Cheong Y. The impact of endometrioma on IVF/ICSI outcomes: a systematic review and meta-analysis. Hum Reprod Update. 2015;21:809–25.
31. Adamson GD, Pasta DJ. Endometriosis fertility index: the new, validated endometriosis staging system. Fertil Steril. 2010;94:1609–15.
32. Tomassetti C, Geysenbergh B, Meuleman C, Timmerman D, Fieuws S, D'Hooghe T. External validation of the endometriosis fertility index (EFI) staging system for predicting non-ART pregnancy after endometriosis surgery. Hum Reprod. 2013;28:1280–8.
33. Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, Heikinheimo O, Horne AW, Kiesel L, Nap A, Prentice A, Saridogan E, Soriano D, Nelen W, European Society of Human Reproduction and Embryology. ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014;29:400–12.
34. Muzii L, Di Tucci C, Di Feliciantonio M, Marchetti C, Perniola G, Panici PB. The effect of surgery for endometrioma on ovarian reserve evaluated by antral follicle count: a systematic review and meta-analysis. Hum Reprod. 2014;29:2190–8.
35. Ferrero S, Venturini PL, Gillott DJ, Remorgida V, Leone Roberti Maggiore U. Hemostasis by bipolar coagulation versus suture after surgical stripping of bilateral ovarian endometriomas: a randomized controlled trial. J Minim Invasive Gynecol. 2012;19:722–30.
36. Muzii L, Achilli C, Bergamini V, Candiani M, Garavaglia E, Lazzeri L, Lecce F, Maiorana A, Maneschi F, Marana R, Perandini A, Porpora M, Seracchioli R, Spangolo E, Vignali M, Benedetti PP. Comparison between the stripping technique and the combined excisional/ablative technique for the treatment of bilateral ovarian endometriomas: a multicentre RCT. Hum Reprod. 2016;31:339–44.
37. Elizur SE, Chian R-C, Holzer HEG, Gidoni Y, Tulandi T, Tan SL. Cryopreservation of oocytes in a young woman with severe and symptomatic endometriosis: a new indication for fertility preservation. Fertil Steril. 2009;91:293.e1–3.
38. Raad J, Sonigo C, Tran C, Sifer C, Cedrin Durnerin I, Grynberg M. Oocyte vitrification for preserving fertility in patients with endometriosis: first observational cohort study and many unresolved questions. Eur J Obstet Gynecol Reprod Biol. 2018;220:140–1.
39. Cobo A, García-Velasco JA. Why all women should freeze their eggs. Curr Opin Obstet Gynecol. 2016;28:206–10.
40. Seyhan A, Urman B, Turkgeldi E, Ata B. Do endometriomas grow during ovarian stimulation for assisted reproduction? A three-dimensional volume analysis before and after ovarian stimulation. Reprod Biomed Online. 2018;36:239–44.

41. Kim SJ, Choo CW, Kim SK, Lee JR, Jee BC, Suh CS, Lee W, Kim S. The effects of letrozole on women with endometriosis undergoing ovarian stimulation for *in vitro* fertilization. Gynecol Endocrinol. 2019;36:257–60.
42. Chen M-J, Yang J-H, Yang Y-S, Ho H-N. Increased occurrence of tubo-ovarian abscesses in women with stage III and IV endometriosis. Fertil Steril. 2004;82:498–9.
43. Yaron Y, Peyser MR, Samuel D, Amit A, Lessing JB. Infected endometriotic cysts secondary to oocyte aspiration for in-vitro fertilization. Hum Reprod. 1994;9:1759–60.
44. Benaglia L, Somigliana E, Iemmello R, Colpi E, Nicolosi AE, Ragni G. Endometrioma and oocyte retrieval–induced pelvic abscess: a clinical concern or an exceptional complication? Fertil Steril. 2008;89:1263–6.
45. von Wolff M, Stute P. Hormonelle Substanzklassen. Gynäkologische Endokrinologie und Reproduktionsmedizin. Schattauer 1. Ausgabe, 2013:5-18.
46. Xue Cao, Hong-yang Chang, Jun-yan Xu, Yi Zheng, Yun-gai Xiang, Bing Xiao, Xu-jing Geng, Li-li Ni, Xi-ying Chu, Shi-bo Tao, Yan He, Gen-hong Mao. The effectiveness of different down-regulating protocols on in vitro fertilization-embryo transfer in endometriosis: a meta-analysis. Reprod Biol and Endocrinol. 2020;18:1.
47. Niu Z, Chen Q, Sun Y, Feng Y. Long-term pituitary downregulation before frozen embryo transfer could improve pregnancy outcomes in women with adenomyosis. Gynecol Endocrinol. 2013;29:1026–30.

Turner Syndrome

Andreas Schüring, Frank Nawroth, and Michael von Wolff

Definition and Symptoms

Turner syndrome (TS) is the name given to various symptoms caused by monosomy X (karyotype 45,X0) (approx. 1/3), monosomy X mosaicism (approx. 1/3) or structural abnormality of an X chromosome (approx. 1/3) [1]. It affects about 1 in 2500 girls [2]. The initial description was made by the paediatrician Ullrich and the gynaecologist Turner.

The severity of the symptoms is usually greater in monosomy X than in mosaicism or structural abnormality.

Gonadal dysgenesis with greatly reduced follicular reserve and premature ovarian insufficiency (POI) as endocrine reproductive characteristics are the focus of the following discussion. Due to the different characteristics of the ovarian reserve in monosomy X compared to mosaicism and structural abnormality, a distinction will be made in this chapter.

A. Schüring
MVZ KITZ Fertility Centre, Regensburg, Germany
e-mail: info@kitz-regensburg.de

F. Nawroth (✉)
Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology, amedes MVZ Hamburg, Hamburg, Germany
e-mail: Frank.Nawroth@amedes-group.com

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_17

Turner Syndrome: Monosomy X

Clinical signs include dwarfism, pterygium colli, renal abnormalities and endocrinopathies. Cardiovascular malformations result in an increased risk of aortic dissection and rupture, associated with significantly increased morbidity and mortality. Due to a lack of or very low ovarian reserve, women with monosomy X are usually infertile.

Turner Syndrome: Mosaicism and Structural Abnormalities

At least 50% of TS patients have mosaicism or a structural chromosomal abnormality. The phenotype is usually less pronounced, and women can also be classified as being prognostically more favourable regarding fertility and pregnancy-associated risks than with monosomy X [3].

At the same time, it is known that genotype-phenotype correlations in patients with TS mosaicism and structural abnormalities cannot be reliably predicted [4, 5]. This is due to a method-dependent diagnostic sensitivity and findings obtained in peripheral lymphocytes or the oral mucosa may differ from other tissues [6]. The occurrence of puberty despite diagnosed monosomy 45,X0 is therefore explained by the presence of a normal cell line in the ovary [7, 8]. Follicles in TS patients may contain genetically normal oocytes, but granulosa cells with monosomy X [9]. It is also postulated that TS patients with monosomy X, which is subject to strong intrauterine genetic selection pressure, must have at least one cryptic “rescue” cell line that has ensured the continuation of their own pregnancy [10]. Furthermore, TS can be modulated by epigenetic effects which are diagnostically inaccessible to karyotyping [11].

Due to the limited predictability of genotype-phenotype correlations, the clinical findings and the ovarian reserve are of central importance when considering possible fertility preservation measures after genetic confirmation of the diagnosis.

Risks of Pregnancy

If fertility preservation measures are considered for a patient with TS, it must be noted that pregnancy in TS patients is associated with increased maternal and foetal morbidity and mortality (Table 1). Even with egg donation, the risk of cardiovascular events and hypertensive complications with their consequences for the mother and foetus is significantly increased [12, 13]. Advice on fertility preservation measures must therefore take into account alongside the associated risks so that the patient can make an informed choice. Screening to identify risk factors and close monitoring of the pregnancy by an interdisciplinary team of experts are necessary.

Table 1 Maternal and foetal pregnancy risks in patients with Turner syndrome

Maternal risks
• Dissection and rupture of the aorta. Risk also increased after pregnancy.
• Hypertension
• Preeclampsia, eclampsia, HELLP syndrome
• Gestational diabetes
• Hypothyroidism
• Caesarean section
Foetal risks
• Miscarriage
• Chromosomal abnormalities
• Preterm birth due to maternal risks (see above)

The data available on the risks depending on the genotype are limited. The genotypic expression correlates with the severity of TS [1], and it can be assumed that this also applies to the relationship between the severity of TS and the risks of pregnancy. Nevertheless, the American Society for Reproductive Medicine (ASRM), which classifies TS as a relative contraindication for pregnancy based on pregnancy outcome, does not distinguish between TS mosaicism and monosomies and considers all patients as being at risk [14–17].

Aortic Dissection and Aortic Rupture

During the volume load of pregnancy, frequent cardiovascular anomalies in TS significantly increase the risk of aortic dissection or rupture. The mortality risk is approximately 2% [12, 16] and is 150 times that of the normal maternal population [18]. In patients with a multiple pregnancy, the risk of aortic dissection increases five-fold [15].

The event itself and the timing of an aortic dissection or rupture cannot be predicted. Predisposing factors besides hypertension are the frequent cardiac abnormalities, especially aortic dilatation, coarctation of the aorta and bicuspid aortic valve [19]. The prevalence of cardiac abnormalities in TS is 25–50% [5]. In cardiac MRI, an aortic size index >2.0 cm/m^3 body surface is associated with a significantly increased risk [20]. Even after pregnancy, the increased risk may persist due to persistent cardiovascular damage [21]. There is no significant association between karyotype and cardiovascular abnormalities in TS, and screening is recommended regardless of genetic findings [5, 14].

Hypertensive Complications and Other Risks

During pregnancy, TS patients have a greatly increased risk of hypertensive disorders along with their complications for mother and foetus. The incidence of hypertensive disorders is up to 67%. They occur as severe manifestations in more than

50% of these cases: preeclampsia, eclampsia and HELLP syndrome [13]. The risk of gestational diabetes and hypothyroidism is also increased [22]. Because of the physical stature, cardiovascular and hypertensive disorders and more frequent intrauterine growth retardation, the caesarean section rate is increased in TS patients [13, 15].

Screening, Risk Advice and Multidisciplinary Monitoring

Every woman with TS who is considering pregnancy must be thoroughly informed about the associated risks. This information should be provided by:

- Cardiologists (specializing in congenital heart disease).
- Prenatal physician/obstetrician in a tertiary centre.
- Human geneticists (when using own oocytes).

The patient should be examined for additional risk factors and associated diseases before the final decision for pregnancy is made (Table 2). During pregnancy, close monitoring by a multidisciplinary team in a tertiary centre is required [14].

Foetal Chromosomal Abnormalities and Miscarriages

Data on foetal chromosomal abnormalities and miscarriages are believed to be largely based on ovarian Turner mosaicism or structural abnormalities of the X chromosome, since women with an X monosomy are usually infertile.

The risk of miscarriage or chromosomal abnormalities is generally increased [17, 23]. Interestingly, miscarriages and malformations have been described not only in spontaneous pregnancies but also in reproductive medicine treatments with donor egg cells [24].

Table 2 Screening before pregnancy in patients with Turner syndrome (mod. [18])

• Cardiologic evaluation (by cardiologist specialised in hereditary heart diseases)
• Echocardiography
• Electrocardiography (ECG)
• Cardiac magnetic resonance imaging (MRI)
• Evaluation of blood pressure
• Thyroid function tests: TSH, free T4, Thyroid antibodies in serum
• Glucose metabolism: Fasting glucose, HbA1c in serum; oral glucose tolerance test
• Liver function tests: γGT, GOT, GPT in serum; liver sonography (if necessary)
• Renal sonography
• Renal function tests in patients with pathological sonography or hypertension
• Vaginal sonography, hysteroscopy (if necessary)
• PAP smear

A review reported 160 pregnancies in 74 women with TS. Miscarriage occurred in 29% of the cases, 20% had a congenital malformation and in 7% of the cases, the pregnancy ended fatally in the perinatal period [23]. Chromosomal abnormalities were found in 24% of the children of 410 TS patients who were identified using a cytogenetic register [25].

Possible causes for the frequent miscarriages in TS patients are quoted: Chromosomal abnormalities [26, 27], uterine malformations and hypoplastic uterus [28], reduced uterine blood flow [26, 29], deficits in endometrial tight junctions [30] and reduced endometrial receptivity due to a 21-dehydroxylase deficiency [31].

Due to the higher rate of foetal chromosomal abnormalities, patients with TS should be informed about prenatal diagnosis or preimplantation genetic testing, PGT [18]. Because of the reduced ovarian reserve, fewer embryos are available for PGT in TS, resulting in lower pregnancy rates. If the oocytes were previously cryopreserved, it is likely that the number available for genetic testing will decrease further.

Fertility

The diagnosis of TS has serious consequences for fertility, which is a major challenge for the patient [26, 32]. The available data show the presence of TS mosaicism and a structural abnormality as a central predictive factor for the reproductive function of TS patients, which, if present, is usually significantly reduced. Only about 30% of TS patients, almost exclusively those with a mosaic karyotype or structural abnormality of the X chromosome, show signs of pubertal development without endocrine therapy. Only 10–20% reach the menarche. The delayed development of the uterus and ovaries was less pronounced in a longitudinal cohort of 38 TS patients with TS mosaicism than with TS monosomy [33]. More than 90% of the women with TS who conceived spontaneously or with the help of reproductive medicine had TS mosaicism [15, 17, 34]. About 90% of all girls with TS lose most or all germ cells before the end of puberty and are permanently infertile.

Fertility Preservation Measures

Based on the above findings on the fertility of TS patients, fertility preservation measures are mainly considered for patients with mosaicism or structural abnormality of the X chromosome. Therefore, the karyotype is of central importance for the indication. The clinical predictive factors must also be considered in the decision (see Table 3), since the genetic findings in the ovary can differ from those in peripheral lymphocytes or the oral mucosa [7].

If fertility preservation is desired, the age of the TS patient is another important decision criterion. It allows a better assessment of the individual prognosis, and

Table 3 Predictive factors for the presence of follicles in patients with Turner syndrome (mod. [36, 37])

• Mosaicism karyotype/structural abnormality of X-Chromosome
• Spontaneous puberty
• Spontaneous menarche
• Normal FSH concentration
• Normal AMH concentration
• Normal antral follicle count (AFC)

according to the current data, an active approach is considered, especially for patients from the age of 14 to 16 years. Active measures are usually not taken before this age. Preservation of ovarian tissue in childhood has been considered [38, 39]. However, due to the unclear later development of TS symptoms at this age and the associated pregnancy risks, as well as the lack of data on the chance of pregnancy, such a procedure is controversially discussed and is considered highly experimental.

In TS patients over the age of 14–16 years, more complex fertility protection measures such as cryopreservation of oocytes or ovarian tissue should be indicated, taking into account the AMH value, menstrual cycle history and the presence of other predictive factors (Table 3) (decision path see Fig. 1).

Cryopreservation of oocytes or ovarian tissue was performed as a fertility-preserving measure as an experimental approach in more than 150 girls and adolescents with TS [40]. Based on the available data, however, the effectiveness of the procedures with regard to later fertility cannot yet be assessed, as there are currently no reports of children being born.

Assessment of the Ovarian Reserve

If pregnancy is fundamentally justifiable and there is endocrine activity in the ovaries, the choice of fertility preservation measure for TS depends on the individual ovarian reserve (Fig. 1).

Lunding et al. [35] examined the predictive value of AMH for spontaneous puberty in prepubertal girls and for POI in adolescents and adults in a study of 120 TS patients. The majority of TS patients with mosaicism had ovarian function in young adulthood. An AMH concentration <4 pmol/L allowed the prognosis that puberty will not occur or that there is a risk of POI in adolescents or adult TS patients.

A Swedish group identified predictive factors for the presence of follicles using laparoscopy in 74 girls with TS, which may be helpful when deciding on a fertility preservation measure [36] (Table 3). Due to the variable genotype-phenotype association of the TS, clinical markers of the ovarian reserve are of particular importance in addition to the genetic findings.

Therefore, complex measures with own germ cells, such as cryopreservation of oocytes or ovarian tissue should be justified by an AMH value well above the detection limit, as well as the simultaneous presence of other factors that allow the prediction of the presence of follicles.

Cryopreservation of Oocytes

The feasibility of oocyte cryopreservation (see chapter "Cryopreservation of Unfertilized and Fertilized Oocytes") has been demonstrated [37, 39, 41]. It should be noted, however, that the women are mostly still young and the medical requirements for cryopreservation of oocytes (transvaginal sonography) must be met in order to offer such a procedure.

The question of predicting the number of oocytes retrieved cannot yet be clearly answered. However, it appears that, in addition to the presence of TS mosaicism or structural abnormality of the X chromosome, the predictive factors AMH, AFC and spontaneous menarche are of particular importance [37, 39, 41].

In a smaller case study in three TS patients with mild phenotypes, but in one case with monosomy X, the AFC, AMH and FSH predicted the ovarian response regardless of the genetic findings [39]. However, Talaulikar et al. [41] found no correlation between AMH and the number of oocytes obtained.

In collectives not affected by TS, it has been shown that depending on age, cryopreservation of oocytes for fertility preservation requires at least 8–10 cells in metaphase II to give the patient a realistic chance of having her own child [42]. Reaching this number of oocytes can be problematic in TS and may require several stimulation cycles. If one considers that due to the limited quality of the oocytes in TS, a significantly higher number of oocytes than in healthy individuals would probably make sense, the dilemma of fertility preservation in TS becomes clear.

Cryopreservation of Ovarian Tissue

The quantity and quality of the egg cells are also important for the later success rates of ovarian tissue cryopreservation (see chapter "Transportation, Cryopreservation and Storage of Ovarian Tissue") [27, 36]. The ethical issues of an invasive intervention in a child who is unable to give consent and the risk of iatrogenic damage to the ovarian reserve as a result of surgery and possible acceleration of POI must also be considered. It is advantageous that menarche is not a prerequisite.

It is recommended that if spontaneous menarche occurs, adolescent TS patients should be presented without delay for a fertility preservation consultation to limit the time-dependent loss of the ovarian reserve [39].

In a study of adolescent TS patients, follicles were found in 60% of the ovarian biopsies. In 78% of these cases, the follicle density was within the 95% confidence interval of a control group. However, a high proportion of abnormal follicular morphology was found [43].

If there is the possibility of cryopreservation of both oocytes and ovarian tissue, cryopreservation of oocytes is recommended. The data available on the effectiveness of transplantation of ovarian tissue (see chapter "Transplantation of Ovarian Tissue") with a low ovarian reserve are very limited. The ovarian reserve should therefore be higher for the cryopreservation of ovarian tissue than for the cryopreservation of oocytes (Fig. 1).

Egg Donation with or without Surrogacy

Egg donation is a way for TC patients to avoid the associated foetal chromosomal risks. However, if she carries the pregnancy herself, the obstetric risk, including significant cardiovascular risks, remains.

In this respect, oocyte donation with surrogate motherhood should be considered as a safer alternative. Only then can all TS-associated risks be excluded. If the patient prefers to carry the pregnancy to term, screening and risk counselling by cardiologists and prenatal physicians are necessary.

It should be noted, however, that egg donation and/or surrogacy are prohibited by law in some countries such as Germany and Switzerland.

Risks of Fertility Preservation Measures

Despite the possible approaches to fertility preservation, it must be borne in mind that TS is not only a difficult entity for fertility preservation in terms of reproductive medicine. Realistically, patients with TS mosaicism or structural chromosomal abnormalities of the X chromosome are particularly suitable for procedures using their own oocytes. The indication should also be based on clinical predictive factors.

TS is also associated with a high risk of morbidity and mortality for pregnant woman. Comprehensive education of the patient, providing differentiated information about the complex situation, is of great importance. In this context, it should be recalled that the ASRM has classified TS as a relative contraindication for pregnancy and recommends surrogacy [14]. Summarising the possible fertility preservation measures, it is clear that simple methods do not completely eliminate the risks associated with TS. Cryopreservation of oocytes or ovarian tissue, egg donation or surrogate motherhood with own eggs are associated with significant risks in TS patients. Only more complex strategies such as egg donation with surrogacy or cryopreservation of oocytes, combined with pre-implantation diagnostics and subsequent surrogacy, can safely avoid the risks associated with the syndrome (Table 4). For this reason, forgoing fertility-protective measures or adoption should also be discussed.

Practical Approach

The many aspects such as the different genotypes, the different manifestations of TS symptoms, the different pregnancy risks, the problem of the limited ovarian reserve and the mostly young age of the girls and women stand in the way of a standard approach when deciding on a fertility preservation measure. The overall situation must be discussed individually, if necessary together with the parents of the often underage girls.

Therefore, the practical procedure can only be roughly outlined (Fig. 1) and the following points mentioned:

Table 4 Risks of different fertility preservation procedures in patients with Turner syndrome

Fertility preservation procedure	Turner syndrome associated risks	
	Maternal and foetal obstetrical risks	Risk of foetal chromosomal anomalies
Freezing of ovarian tissue	Risk not eliminated	Risk not eliminated
Freezing of oocytes	Risk not eliminated	Risk not eliminated
Oocyte donation	Risk not eliminated	Risk eliminated
Surrogacy[a] with own oocytes	Risk eliminated	Risk not eliminated
Surrogacy[a] with donor oocytes[a]	Risk eliminated	Risk eliminated
Freezing of oocytes, preimplantation genetic testing (PGT), surrogacy[a]	Risk eliminated	Risk eliminated
Freezing of ovarian tissue, PGT, surrogacy[a]	Risk eliminated	Risk eliminated
No procedure, adoption of children	Risk eliminated	Risk eliminated

[a]Prohibited in some countries such as Germany and Switzerland

1. A fertility preservation measure only makes sense if it can be assumed that a later pregnancy is possible in terms of health.
2. The first important differentiation for the prognosis is the distinction between TS monosomy and TS mosaic or a structural abnormality of the X chromosome. However, fertility preservation is – independently of this – useful if clinical predictive factors indicate the presence of an adequate ovarian reserve. Since the correlation between ovarian reserve and corresponding markers varies, stimulation experiments or ovarian biopsy and histological diagnosis prior to cryopreservation are possible strategies.
3. If the ovarian reserve is still preserved, it may be possible to monitor AMH and wait, as a preserved ovarian reserve can remain for years in young women [35].
4. Cryopreservation of ovarian tissue may be preferred in a virgin. If oocytes are to be cryopreserved, follicle aspiration under general anaesthesia with a small vaginal probe is required.
5. With regard to ovarian stimulation, given the expected severely limited ovarian reserve, administration of high-dose gonadotropin as a standard approach makes the most sense based on current data, if there is a realistic chance of obtaining several oocytes from the patient [37]. In unfavourable low-responder situations, low-dose stimulation or aspiration during the natural cycle may be justified.
6. Pregnancies after cryopreservation of oocytes or ovarian tissue in TS patients have not yet been reported and the method is considered experimental [40, 44]. Because of the chromosomal risks associated with procedures using the patient‘s own oocytes, prenatal diagnostic procedures should be addressed, and in the case of an in vitro procedure, pre-implantation diagnostics.
7. The transfer of only one embryo is preferred because a multiple pregnancy increases the primary risk of cardiovascular complications (aortic dissection) fivefold [15]. The small body size of a TS patient and the resulting obstetric problems with a multiple pregnancy must also be considered.

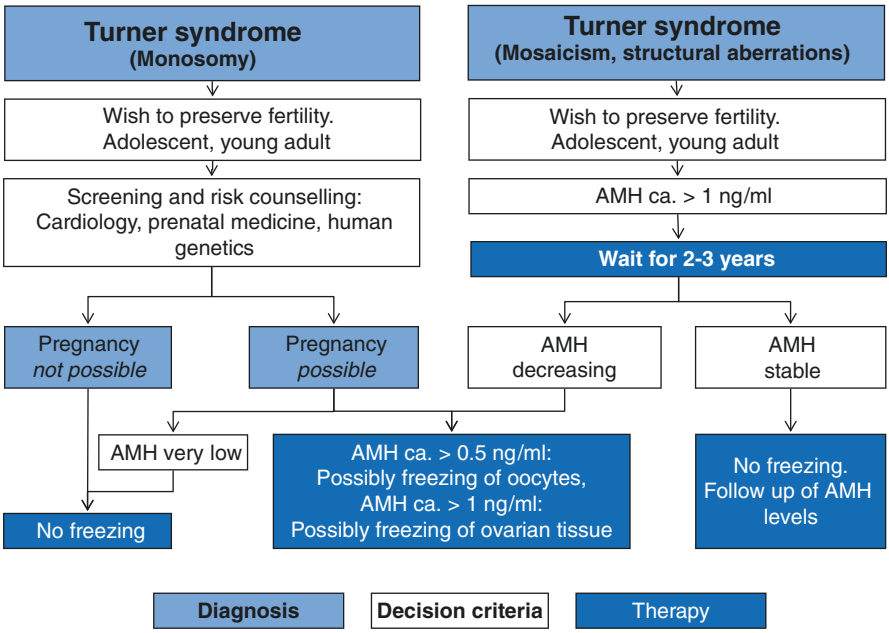


Fig. 1 Treatment decision flowchart for fertility preservation procedures in patients with TS

References

1. Zheng J, Liu Z, Xia P, Lai Y, Wei Y, Liu Y, Chen J, Qin L, Xie L, Wang H. Clinical manifestation and cytogenetic analysis of 607 patients with Turner syndrome. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2017;34:61–4.
2. Sybert VP, McCauley E. Turner's syndrome. N Engl J Med. 2004;351:1227–38.
3. Jacobs P, Dalton P, James R, Mosse K, Power M, Robinson D, Skuse D. Turner syndrome: a cytogenetic and molecular study. Ann Hum Genet. 1997;61:471–83.
4. Miguel-Neto J, Carvalho AB, Marques-de-Faria AP, Guerra-Júnior G, Maciel-Guerra AT. New approach to phenotypic variability and karyotype-phenotype correlation in Turner syndrome. J Pediatr Endocrinol Metab. 2016;29:475–9.
5. Noordman I, Duijnhouwer A, Kapusta L, Kempers M, Roeleveld N, Schokking M, Smeets D, Freriks K, Timmers H, van Alfen-van der Velden J. Phenotype in girls and women with Turner syndrome: Association between dysmorphic features, karyotype and cardio-aortic malformations. Eur J Med Genet. 2018;61:301–6.
6. Yang S. Diagnostic and therapeutic considerations in Turner syndrome. Ann Pediatr Endocrinol Metab. 2017;22:226–30.
7. Balen AH, Harris SE, Chambers EL, Picton HM. Conservation of fertility and oocyte genetics in a young woman with mosaic Turner syndrome. BJOG. 2010;117:238–42.
8. Grynberg M, Bidet M, Benard J, Poulain M, Sonigo C, Cédrin-Durnerin I, Polak M. Fertility preservation in Turner syndrome. Fertil Steril. 2016;105:13–9.
9. Peek R, Schleedoorn M, Smeets D, van de Zande G, Groenman F, Braat D, van der Velden J, Fleischer K. Ovarian follicles of young patients with Turner's syndrome contain normal oocytes but monosomic 45,X granulosa cells. Hum Reprod. 2019;34:1686–96.

10. Hook EB, Warburton D. Turner syndrome revisited: review of new data supports the hypothesis that all viable 45,X cases are cryptic mosaics with a rescue cell line, implying an origin by mitotic loss. Hum Genet. 2014;133:417–24.
11. Álvarez-Nava F, Lanes R. Epigenetics in Turner syndrome. Clin Epigenetics. 2018;10:45.
12. Karnis MF, Zimon AE, Lalwani SI, Timmreck LS, Klipstein S, Reindollar RH. Risk of death in pregnancy achieved through oocyte donation in patients with Turner syndrome: a national survey. Fertil Steril. 2003;80:498–501.
13. Bodri D, Vernaeve V, Figueras F, Vidal R, Guillén JJ, Coll O. Oocyte donation in patients with Turner's syndrome: a successful technique but with an accompanying high risk of hypertensive disorders during pregnancy. Hum Reprod. 2006;21:829–32.
14. Practice Committee of American Society for Reproductive Medicine. Increased maternal cardiovascular mortality associated with pregnancy in women with Turner syndrome. Fertil Steril. 2012;97:282–4.
15. Hadnott TN, Gould HN, Gharib AM, Bondy CA. Outcomes of spontaneous and assisted pregnancies in Turner syndrome: the U.S. National Institutes of Health experience. Fertil Steril. 2011;95:2251–6.
16. Chevalier N, Letur H, Lelannou D, Ohl J, Cornet D, Chalas-Boissonnas C, Frydman R, Catteau-Jonard S, Greck-Chassain T, Papaxanthos-Roche A, Dulucq MC, Couet ML, Cédrin-Durnerin I, Pouly JL, Fénichel P, French Study Group for Oocyte Donation. Materno-fetal cardiovascular complications in Turner syndrome after oocyte donation: insufficient prepregnancy screening and pregnancy follow-up are associated with poor outcome. J Clin Endocrinol Metab. 2011;96:E260–7.
17. Bryman I, Sylvén L, Berntorp K, Innala E, Bergström I, Hanson C, Oxholm M, Landin-Wilhelmsen K. Pregnancy rate and outcome in Swedish women with Turner syndrome. Fertil Steril. 2011;95:2507–10.
18. Karnis MF. Fertility, pregnancy, and medical management of Turner syndrome in the reproductive years. Fertil Steril. 2012;98:787–91.
19. Carlson M, Silberbach M. Dissection of the aorta in Turner syndrome: two cases and review of 85 cases in the literature. J Med Genet. 2007;44:745–9.
20. Matura LA, Ho VB, Rosing DR, Bondy CA. Aortic dilatation and dissection in Turner syndrome. Circulation. 2007;116:1663–70.
21. Gravholt CH, Landin-Wilhelmsen K, Stochholm K, Hjerrild BE, Ledet T, Djurhuus CB, Sylvén L, Baandrup U, Kristensen BØ, Christiansen JS. Clinical and epidemiological description of aortic dissection in Turner's syndrome. Cardiol Young. 2006;16:430–6.
22. Bondy CA, Turner Syndrome Study Group. Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. J Clin Endocrinol Metab. 2007;92:10–25.
23. Tarani L, Lampariello S, Raguso G, Colloridi F, Pucarelli I, Pasquino AM, Bruni LA. Pregnancy in patients with Turner's syndrome: six new cases and review of literature. Gynecol Endocrinol. 1998;12:83–7.
24. Foudila T, Söderström-Anttila V, Hovatta O. Turner's syndrome and pregnancies after oocyte donation. Hum Reprod. 1999;14:532–5.
25. Birkebaek NH, Crüger D, Hansen J, Nielsen J, Bruun-Petersen G. Fertility and pregnancy outcome in Danish women with Turner syndrome. Clin Genet. 2002;61:35–9.
26. Abir R, Fisch B, Nahum R, Orvieto R, Nitke S, Ben RZ. Turner's syndrome and fertility: current status and possible putative prospects. Hum Reprod Update. 2001;7:603–10.
27. Hreinsson JG, Otala M, Fridström M, Borgström B, Rasmussen C, Lundqvist M, Tuuri T, Simberg N, Mikkola M, Dunkel L, Hovatta O. Follicles are found in the ovaries of adolescent girls with Turner's syndrome. J Clin Endocrinol Metab. 2002;87:3618–23.
28. Yaron Y, Ochshorn Y, Amit A, Yovel I, Kogosowki A, Lessing JB. Patients with Turner's syndrome may have an inherent endometrial abnormality affecting receptivity in oocyte donation. Fertil Steril. 1996;65:1249–52.
29. Hovatta O. Pregnancies in women with Turner's syndrome. Ann Med. 1999;31:106–10.
30. Rogers PA, Murphy CR, Leeton J, Hoise MJ, Beaton L. Turner's syndrome patients lack tight junctions between uterine epithelial cells. Hum Reprod. 1992;7:883–5.

31. Cohen MA, Sauer MV, Lindheim SR. 21-Hydroxylase deficiency and Turner's syndrome: a reason for diminished endometrial receptivity. Fertil Steril. 1999;72:937–9.
32. Sutton EJ, McInerney-Leo A, Bondy CA, Gollust SE, King D, Biesecker B. Turner syndrome: four challenges across the lifespan. Am J Med Genet. 2005;139:57–66.
33. Mazzanti L, Cacciari E, Bergamaschi R, Tassinari D, Magnani C, Perri A, Scarano E, Pluchinotta V. Pelvic ultrasonography in patients with Turner syndrome: age-related findings in different karyotypes. J Pediatr. 1997;131(1 Pt 1):135–40.
34. Baudier MM, Chihal HJ, Dickey RP. Pregnancy and reproductive function in a patient with non-mosaic Turner syndrome. Obstet Gynecol. 1985;65(3 Suppl):60S–4S.
35. Lunding SA, Aksglaede L, Anderson RA, Main KM, Juul A, Hagen CP, Pedersen AT. AMH as predictor of premature ovarian insufficiency: a longitudinal study of 120 Turner syndrome patients. J Clin Endocrinol Metab. 2015;100:E1030–8.
36. Borgström B, Hreinsson J, Rasmussen C, Sheikhi M, Fried G, Keros V, Fridström M, Hovatta O. Fertility preservation in girls with Turner syndrome: prognostic signs of the presence of ovarian follicles. J Clin Endocrinol Metab. 2009;94:74–80.
37. Oktay K, Bedoschi G. Oocyte cryopreservation for fertility preservation in postpubertal female children at risk for premature ovarian failure due to accelerated follicle loss in Turner syndrome or cancer treatments. J Pediatr Adolesc Gynecol. 2014;27:342–6.
38. Oktay K, Bedoschi G, Berkowitz K, Bronson R, Kashani B, McGovern P, Pal L, Quinn G, Rubin K. Fertility preservation in women with Turner syndrome: a comprehensive review and practical guidelines. J Pediatr Adolesc Gynecol. 2016;29:409–16.
39. Vergier J, Bottin P, Saias J, Reynaud R, Guillemain C, Courbiere B. Fertility preservation in Turner syndrome: Karyotype does not predict ovarian response to stimulation. Clin Endocrinol (Oxf). 2019;91:646–51.
40. Schleedorn MJ, van der Velden AAEM, Braat DDM, Peek R, Fleischer K. To freeze or not to freeze? An update on fertility preservation in females with Turner syndrome. Pediatr Endocrinol Rev. 2019:369–82.
41. Talaulikar VS, Conway GS, Pimblett A, Davies MC. Outcome of ovarian stimulation for oocyte cryopreservation in women with Turner syndrome. Fertil Steril. 2019;111:505–9.
42. Cobo A, García-Velasco JA, Coello A, Domingo J, Pellicer A, Remohí J. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril. 2016;105:755–64.e8.
43. Mamsen LS, Charkiewicz K, Anderson RA, Telfer EE, McLaughlin M, Kelsey TW, Kristensen SG, Gook DA, Ernst E, Andersen CY. Characterization of follicles in girls and young women with Turner syndrome who underwent ovarian tissue cryopreservation. Fertil Steril. 2019;111:1217–25.e3.
44. Oktay K, Bedoschi G. Fertility preservation in girls with Turner syndrome: limitations, current success and future prospects. Fertil Steril. 2019:1124–6.

Transgender

Kenny A. Rodriguez-Wallberg

Introduction

People who identify themselves as transgender or gender nonconforming experience internal psychological conflict due to incongruence between the gender assigned at birth, and the gender in which the individual identifies [1]. The distress experienced by gender incongruence is known as gender dysphoria. Many of these patients choose to undergo treatments to alleviate the distress associated with gender dysphoria [2, 3]. These treatments are aimed at the suppression of assigned gender sexual characteristics through medical gender-affirming hormone treatment (GAHT) and sex reassignment surgery (SRS) in many cases. Table 1 summarises the glossary of terms and definitions.

Standards of care for the health of transsexual, transgender and gender-nonconforming people have been provided by the World Professional Association for Transgender Health (WPATH) [1]. In the WPATH guidelines, it is recommended that all transgender individuals receive appropriate counselling on the potential negative impact of GAHT and SRS on future fertility possibilities, as well as counselling on fertility preservation options [1, 4]. These current recommendations are supported by large reproductive medical societies such as the American Society of Reproductive Medicine, ASRM [5], and the European Society of Human Reproduction and Embryology, ESHRE [6]. However, the costs of the procedures become a major barrier preventing young transgender patients from undergoing fertility preservation, and only a few countries have been reported to cover these procedures in transgender patients [7, 8].

K. A. Rodriguez-Wallberg (✉)
Department of Reproductive Medicine and Oncology-Pathology, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden
e-mail: kenny.rodriguez-wallberg@sll.se

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_18

Table 1 Summary of definitions currently applied in the field of transgender medicine

Assigned sex at birth[a]	The assigned sex at birth typically based on primary sex characteristics.
Cisgender[a]	A term for a person whose gender identity matches the gender they were assigned at birth; someone who is not trans.
Cisnormative/ cisnormativity[a]	The assumption in individuals or in institutions that everyone is cisgender (no trans).
Gender dysphoria	Distress that gender incongruence might cause.
Gender identity	The gender one identifies with, such as man or woman or an alternative gender, regardless of assigned sex at birth.
Gender incongruence	When a person's gender identity and/or expression do not align with their sex assigned at birth.
Transition[b]	Period of time when individuals change from the gender role associated with their sex assigned at birth to a different gender role. For many people this involves learning how to live socially in another gender role; for others this means finding a gender role and expression that is most comfortable for them. Transition may or may not include feminization or masculinization of the body through hormones or other medical procedures. The nature and duration of transition are variable and individualized.
Transgender[b]	Adjective to describe a diverse group of individuals who cross or transcend culturally defined categories of gender. The gender identity of transgender people differs to varying degrees from the sex they were assigned at birth.
Transgender man:	
Female to male (FtM)	Adjective to describe individuals assigned female at birth who are changing or who have changed their body and/or gender role from birth-assigned female to a more masculine body or role.
Transgender woman:	
Male to female (MtF)	Adjective to describe individuals assigned male at birth who are changing or who have changed their body and/or gender role from birth-assigned male to a more feminine body or role.
Transsexual[c]	Transsexualism F64.0: a medical diagnosis (ICD-10): "A desire to live and be accepted as a member of the opposite sex, usually accompanied by a sense of discomfort with, or inappropriateness of, one's anatomic sex, and a wish to have surgery and hormonal treatment to make one's body as congruent as possible with one's preferred sex, so-called gender dysphoria".[c] The ICD-10 diagnosis is needed in many countries to access gender-affirming health care. In some countries, the diagnosis is called Gender Identity Disorder (DSM-IV or Gender Dysphoria (DSM-5)). A revised new diagnosis with proposed new name "Gender Incongruence" is expected to appear in the upcoming ICD-11.
Transition[b]	Period of time when individuals change from the gender role associated with their sex assigned at birth to a different gender role. The nature and duration of transition are variable and individualized.

[a]Definition copied verbatim from James-Abra et al. 2015 [15]
[b]Definition copied verbatim from WPATH Standards of Care [1]
[c]Definition copied verbatim from ICD-10, F64.0 (World Health Organization, 2016)

Fertility

The treatment of transgender persons is complex and multidisciplinary teamwork is needed. In many centres worldwide, the diagnosis is established and treatments are provided within multidisciplinary gender teams usually dedicated to either adults or younger adolescents, including endocrinologists, psychiatrists, psychologists, plastic surgeons and several other specialists.

It has been difficult to establish the prevalence of transgender or gender-nonconforming people. Studies in the USA report on 0.6% of the population and an increase in referrals and people who receive GAHT [9].

When patients initiate GAHT at a young age, the future of their fertility potential is of concern. It has been estimated that about half of transgender people may have a desire to have children sometime in their life. However, at the time of transition, the number of patients who express this desire is somewhat lower at less than 25% [10]. It is important to consider that many individuals have acknowledged that their feelings about having biological children could change in the future and they may regret not having pursued fertility preservation [11].

Effect of GAHT on Fertility Potential

Long-time effects of GAHT on fertility have been reported by several studies. A recent meta-analysis identified nine studies evaluating the effect of GAHT on semen parameters of transgender women who had undergone estrogen treatment for variable periods of time. Studies found a high proportion of sperm abnormalities, such as oligospermia, teratozoospermia and asthenozoospermia in samples from individuals who had initiated GAHT treatment [7]. Studies of post-orchidectomy specimens 5–8 years after GAHT with estrogen used with or without antiandrogens evidenced abnormal spermatogenesis, maturation arrest or absence of spermatogenesis and tissue fibrosis among individuals who had completed longer treatment [7]. The studies also suggest that the combination of estrogen and GnRH agonists (GnRHa), or estrogen and anti-androgens, inhibit spermatogenesis to a higher degree than the use of estrogen alone [7].

Studies of GAHT effects on transgender men regarding ovarian function have reported a reduction in AMH levels following GnRHa treatment and further reduction following androgenic treatment [12]. Four studies compiled in a meta-analysis by Baram et al. [7] indicate variable ovarian histology results, from preserved ovarian tissue morphology to abnormalities such as large proportions of atretic follicles, thicker ovarian cortex, hyperplastic collagen, ovarian stromal hyperplasia and stromal luteinisation [13].

Data on pregnancies and live births in individuals who have undergone gender transition are scarce. Case reports and a few case series are available, as

summarized by Baram et al. [7]. Data from transgender women who have used their cryopreserved sperm for insemination or IVF/ICSI of a cis-gender partner have been reported. Similarly, data on transgender men who have attempted pregnancy after discontinuation of GAHT are available and naturally occurring pregnancies have been reported. In a study of 41 transgender men, 68% discontinued treatment in order to achieve pregnancy and 20% conceived while still amenorrhoeic from testosterone treatment [14].

Counselling on Fertility Preservation

Although the reproductive needs of the transgender population have become more recognized during recent years and programs for fertility preservation have been established at numerous centres worldwide, studies show high variation in reproductive counselling provision to transgender patients, with higher rates at specialised centres having gender teams or an endocrinologist experienced in fertility treatment, but low rates otherwise [15].

It is important that the centres providing care to transgender patients, including reproductive counselling and fertility preservation, have competence regarding transgender medicine. This is due to evidence that transgender patients are vulnerable when considering fertility preservation [8]. The information should be provided to transgender individuals in a sensitive way, considering their gender dysphoria. Transgender individuals are interested in receiving information about cells which could potentially be used in the future to achieve a pregnancy, i.e. oocytes or sperm, but they prefer not to be reminded of the feminine association of oocytes, or the masculine association of sperm. The language routinely used at reproductive centres usually includes gender-specific words, such as woman, female, vagina, womb, and men, male, testis, penis. These word examples are accepted within a cis-patient population. However, transgender individuals may consider these words offensive.

An important consideration is the use of the correct pronoun used by the individual. The individual should be asked what their preferred pronoun is (he, him, his; she, her, hers) and this should be documented in the patient's chart to ensure that all personnel at the centre use it, guaranteeing that the individual feels recognised and respected by the healthcare professionals [8]. For most patients, non-gendered terms such as patient, person, individual, which are gender-neutral, are well accepted and should be preferred.

Information provision should allow patients to make an informed decision about their future fertility chances and should not implicitly discourage them in any way, as reported by a great number of transgender patients in a recent Australian study [16]. Healthcare professionals counselling on fertility preservation should provide complete information and support to ensure that transgender patients interested in preserving fertility may take that opportunity.

Fertility Preservation

Individuals who are referred for fertility preservation may have already initiated hormonal gender-affirming treatment. If these patients aim to cryopreserve sperm or oocytes, the affirming treatment needs to be suspended. Some transgender women may require several months to resume natural sperm production after suspending estrogenic treatment, similarly to transgender men resuming menses after suspending depo-testosterone. A timely consultation for fertility preservation should be planned before the initiation of gender-affirming hormonal treatment.

Oocyte Cryopreservation

Transgender men should receive information on methods of fertility preservation, and their clinical or experimental status. In general, it is important to clearly explain to transgender men where the oocytes are, why the ovaries need to undergo hormonal stimulation to obtain mature oocytes and that retrieval of the oocytes is feasible through transvaginal puncture of the ovaries. The current medical illustrations used to explain these facts to cis-patients may be inappropriate to transgender patients, as they may negatively react to pictures which remind them of a feminine body [8]. People with gender dysphoria may not have previously undergone a vaginal examination or a transvaginal ultrasound examination. The need for vaginal examination using transvaginal ultrasound should be clearly explained. Some patients may feel discouraged or hesitant about undergoing a vaginal examination during the first medical visit or may need repeated attempts to accomplish it. A number of patients may never undergo a vaginal examination.

Young patients usually have a high ovarian reserve and treatment with exogen gonadotropins to obtain multiple follicles is usually effective in such patients. Older patients may require higher gonadotropin doses. An estimation of the individual's reserve using anti-Müllerian hormone to evaluate the specific chances of obtaining oocytes that can be cryopreserved may provide useful information. A transvaginal ultrasound is also needed before planning treatment, mainly to examine the feasibility of ovarian stimulation aimed at oocyte freezing.

In Sweden, programs for fertility preservation initiated the care of transgender patients in 2013 after a change in the law that finally removed the previous requirement of sterilization. At our centre at Karolinska University Hospital in Stockholm, we use a stimulation protocol incorporating an aromatase inhibitor (letrozole) alongside gonadotropin stimulation for fertility preservation aiming at oocyte freezing in transgender men. This protocol, initially developed for women with estrogen-sensitive breast cancer requiring fertility preservation, significantly reduces the systemic estradiol rise during stimulation and minimizes estrogenic side effects [17]. The protocol using letrozole has been reported in the specific clinical situation of transgender men undergoing ovarian stimulation with good patient adherence [18].

Sperm Cryopreservation

The biological features and the continuous production of mature sperm since puberty might be an advantage for transgender women interested in sperm banking. Transgender women should be encouraged to provide a sperm sample for sperm banking as soon as possible before gender-affirming treatment with opposing hormones is initiated. If sperm is banked, the chances of success after using frozen-thawed sperm in future fertility treatments are high. Most transgender women who have not initiated gender-affirming treatment are able to have erection and ejaculate. There are usually many spermatozoa in the ejaculate, several million in the majority of cases, so if it is possible for the patient to give a semen sample, the chances of fertility preservation are reasonably high in this group.

Gender-affirming hormone therapy for transgender women consists mainly of estrogen, often combined with an anti-androgen to further suppress testosterone production. This treatment has a known negative effect on the patient's fertility, since the testicles atrophy and serum levels of testosterone drop significantly causing sperm production to halt [19, 20]. Transgender women who are already undergoing estrogen treatment and are interested in recovering sperm to cryopreserve may need to suspend their treatment for several months before sperm production resumes.

For transgender women interested in sperm banking, the logistics are less problematic. Patients provide a semen sample by masturbation to the reproductive laboratory. In crowded centres, transgender women should receive appointments to provide sperm samples to the laboratory at times when cis-male patients are not usually scheduled to provide sperm samples.

Data on Fertility Preservation

A few studies investigating fertility preservation in transgender individuals have been reported, with most of the data investigating patient experiences using qualitative research methods.

In a Belgian cohort of 50 transgender men, half reported a wish to have children, and if fertility preservation options had been available at the time of their transition, 38% would have considered using them [21].

Whereas clinical experiences of sperm banking among transgender women have been reported [22], only a few case reports of transgender men who have undergone fertility preservation by ovarian stimulation and oocyte cryopreservation are available [18, 23].

A study of transgender men's experiences of fertility preservation through oocyte freezing found an increase in gender dysphoria in connection with the procedures involved, such as transvaginal examination and hormonal stimulation [8]. The individuals used several coping strategies to manage the procedures, such as focusing

on their reasons for undergoing fertility preservation [8]. A Canadian study of nine transgender individuals' experiences of assisted reproduction services showed overall negative experiences of healthcare encounters, such as having to cope with normative assumptions and being refused service [15]. A large Australian study of 409 transgender and non-binary adults found that participants with positive experiences of FP often described healthcare professionals who were professional and knowledgeable and provided affirming and caring services, whereas negative experiences were associated with healthcare encounters with professionals who acted mainly as gatekeepers of fertility preservation [16]. Thus, healthcare providers may be encouraging or discouraging towards fertility preservation. The mentioned Australian study discusses the fact that although the WPATH Standards of Care published in 2011 [24] do address the topic of fertility preservation, only brief information is provided, and several specific issues are not covered in the guidelines.

The experience of our centre in Sweden and reported patient experiences [8] indicate that it is important to ensure healthcare professionals' continued education so that they can encounter a new patient group. Healthcare professionals' providing care to a new patient group of transgender people at our centre have also reported challenging experiences [25]. A major challenge for optimal care is attainment of good communication and confrontation with preconceived opinions and cis-normative assumptions [26].

For transgender men, it may be expected that ovarian stimulation and transvaginal examinations are likely to increase distress and gender dysphoria, and patients should receive information on this increased risk [8]. It is also important that healthcare providers use non-gendered words as far as possible, such as "bleeding" or "pelvic examination" instead of "menstruation" or "gynaecological examination", and to use the individual's preferred pronoun [8]. Information brochures should be specifically adapted to transgender patients to clearly explain the procedures needed and what to expect.

Table 2 shows strategies coping with the distress of fertility preservation procedures reported by transgender men [8]. These methods of handling the situation, such as focusing on the goal, enlisting support from friends or relatives or using distractors during the examinations should be discussed with the patients. Contextual

Table 2 Experiences of transgender men undergoing fertility preservation (FP) by ovarian stimulation and oocyte cryopreservation in Sweden. Content analysis of individual in-depth qualitative interviews allowed identification of main categories and subcategories [8]

The journey of fertility preservation	Reactions to the fertility preservation proceedings	Strategies for coping
Referral, assessment, diagnosis	Discontinuing testosterone treatment to regain menstruation	Goal-oriented
A frustrating wait	Resumption of menstruation	Searching for support
	The hormonal treatment	Changing the focus
	Becoming exposed by pelvic examinations and being seen by others	A cognitive approach
Doubts and encouragement	Not as bad as anticipated	

sensitivity during fertility preservation procedures is important, and healthcare providers should have knowledge of transgender patients' vulnerable situation in connection with fertility preservation. With this knowledge, providers can help to reduce distress through their actions, or at least not increase it.

Practical Recommendations

- It is of great importance that the information provided to transgender persons is communicated by healthcare professionals in a sensitive way, ideally by healthcare providers with expertise in fertility preservation and with knowledge of transgender medicine.
- The information should also be adapted to the individual's age and it should be accurate, including the shortcomings of current fertility preservation methods, as none of the methods available guarantee a pregnancy and a live birth through the use of cryopreserved cells or tissues that are thawed and revitalized to be used in developed ART treatments.

References

1. Coleman E, Bockting W, Botzer M, Cohen-Kettenis P, De Cuypere G, Feldman J, Fraser L, Green J, Knudson G, Meyer WJ, Monstrey S, Adler RK, Brown GR, Devor AH, Ehrbar R, Ettner R, Eyler E, Garofalo R, Karasic DH, Lev AI, Mayer G, Meyer-Bahlburg H, Hall BP, Pfaefflin F, Rachlin K, Robinson B, Schechter LS, Tangpricha V, van Trotsenburg M, Vitale A, Winter S, Whittle S, Wylie KR, Zucker K. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. Int J Transgend. 2012;13:165–232.
2. Meriggiola MC, Gava G. Endocrine care of transpeople part I. a review of cross-sex hormonal treatments, outcomes and adverse effects in transmen. Clin Endocrinol. 2015;83:597–606.
3. T'Sjoen G, Arcelus J, Gooren L, Klink DT, Tangpricha V. Endocrinology of transgender medicine. Endocr Rev. 2019;40:97–117.
4. Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, Rosenthal SM, Safer JD, Tangpricha V, T'Sjoen GG. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2017;102:3869–903.
5. Ethics Committee of the American Society for Reproductive Medicine. Access to fertility services by transgender persons: an ethics committee opinion. Fertil Steril. 2015;104:1111–5.
6. De Wert G, Dondorp W, Shenfield F, Barri P, Devroey P, Diedrich K, Tarlatzis B, Provost V, Pennings G. ESHRE task force on ethics and law 23: medically assisted reproduction in singles, lesbian and gay couples, and transsexual peopledagger. Hum Reprod. 2014;29:1859–65.
7. Baram S, Myers SA, Yee S, Librach CL. Fertility preservation for transgender adolescents and young adults: a systematic review. Hum Reprod Update. 2019;25:694–716.
8. Armuand G, Dhejne C, Olofsson JI, Rodriguez-Wallberg KA. Transgender men's experiences of fertility preservation: a qualitative study. Hum Reprod. 2017;32:383–90.
9. Crissman HP, Berger MB, Graham LF, Dalton VK. Transgender demographics: a household probability sample of US adults, 2014. Am J Public Health. 2017;107:213–5.

10. Auer MK, Fuss J, Nieder TO, Briken P, Biedermann SV, Stalla GK, Beckmann MW, Hildebrandt T. Desire to have children among transgender people in Germany: a cross-sectional multicenter study. J Sex Med. 2018;15:757–67.
11. Strang JF, Jarin J, Call D, Clark B, Wallace GL, Anthony LG, Kenworthy L, Gomez-Lobo V. Transgender youth fertility attitudes questionnaire: measure development in nonautistic and autistic transgender youth and their parents. J Adolesc Health. 2018;62:128–35.
12. Caanen MR, Soleman RS, Kuijper EA, Kreukels BP, De Roo C, Tilleman K, De Sutter P, van Trotsenburg MA, Broekmans FJ, Lambalk CB. Antimüllerian hormone levels decrease in female-to-male transsexuals using testosterone as cross-sex therapy. Fertil Steril. 2015;103:1340–5.
13. De Roo C, Lierman S, Tilleman K, Peynshaert K, Braeckmans K, Caanen M, Lambalk CB, Weyers S, T'Sjoen G, Cornelissen R, De Sutter P. Ovarian tissue cryopreservation in female-to-male transgender people: insights into ovarian histology and physiology after prolonged androgen treatment. Reprod Biomed Online. 2017;34:557–66.
14. Light A, Wang LF, Zeymo A, Gomez-Lobo V. Family planning and contraception use in transgender men. Contraception. 2018;98:266–9.
15. James-Abra S, Tarasoff LA, Green D, Epstein R, Anderson S, Marvel S, Steele LS, Ross LE. Trans people's experiences with assisted reproduction services: a qualitative study. Hum Reprod. 2015;30:1365–74.
16. Bartholomaeus C, Riggs DW. Transgender and non-binary Australians' experiences with healthcare professionals in relation to fertility preservation. Cult Health Sex. 2019;18:1–17.
17. Oktay K, Turkcuoglu I, Rodriguez-Wallberg KA. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. Reprod Biomed Online. 2010;20:783–8.
18. Rodriguez-Wallberg KA, Dhejne C, Stefenson M, Degerblad M, Olofsson JI. Preserving eggs for men's fertility. A pilot experience with fertility preservation for female-to-male transsexuals in Sweden. Fertil Steril. 2014;102:E160–1.
19. Sapino A, Pagani A, Godano A, Bussolati G. Effects of estrogens on the testis of transsexuals: a pathological and immunocytochemical study. Virchows Arch A Pathol Anat Histopathol. 1987;411:409–14.
20. Jindarak S, Nilprapha K, Atikankul T, Angspatt A, Pungrasmi P, Iamphongsai S, Promniyom P, Suwajo P, Selvaggi G, Tiewtranon P. Spermatogenesis abnormalities following hormonal therapy in transwomen. Biomed Res Int. 2018;2018:7919481.
21. Wierckx K, Van Caenegem E, Pennings G, Elaut E, Dedecker D, Van de Peer F, Weyers S, De Sutter P, T'Sjoen G. Reproductive wish in transsexual men. Hum Reprod. 2012;27:483–7.
22. Wierckx K, Stuyver I, Weyers S, Hamada A, Agarwal A, De Sutter P, T'Sjoen G. Sperm freezing in transsexual women. Arch Sex Behav. 2012;41:1069–71.
23. Wallace SA, Blough KL, Kondapalli LA. Fertility preservation in the transgender patient: expanding oncofertility care beyond cancer. Gynecol Endocrinol. 2014;30:868–71.
24. World Professional Association for Transgender Health (WPATH). Standards of care for the health of transsexual, transgender, and gender nonconforming people, 2011; version 7. https://www.wpath.org/media/cms/Documents/SOC%20v7/Standards%20of%20Care_V7%20Full%20Book_English.pdf.
25. Armuand G, Falck F, Dhejne C, Rodriguez-Wallberg KA. Learning the needs of a new patient group: Health care professionals experiences of caring for transgender men undergoing fertility preservation by cryopreservation of oocytes. In European Professional Association of Transgender Health, EPATH 2019, Inside matters, abstract book 2019: 306. https://epath.eu/wp-content/uploads/2019/04/Boof-of-abstracts-EPATH2019.pdf.
26. Erbenius T, Gunnarsson PJ. Unlearning cisnormativity in the clinic: enacting transgender reproductive rights in everyday patient encounters. J Int Women Stud. 2018;20:27–39.

Part III
Fertility Preservation—Techniques

Ovarian Stimulation to Collect Oocytes

Ariane Germeyer and Michael von Wolff

Introduction

The aim of ovarian stimulation is to obtain mature oocytes in order to preserve them in an unfertilised or fertilised state as a fertility reserve. In the *Ferti*PROTEKT network, stimulation for the collection and cryopreservation of unfertilised or fertilised oocytes was carried out in approximately 40% of all fertility-preserving therapies performed [1].

The distinctive features of this form of fertility preservation are, on the one hand, that the treatment should be carried out as quickly as possible, so that necessary treatment for the underlying disease is not unnecessarily delayed. On the other hand, only one attempt is usually possible, which must be as efficient as possible. The maximum possible number of mature oocytes should be obtained without the risk of overstimulation.

It is also important to note that fertilised eggs can only be transferred to the woman with the consent of both partners. Since the cells can be stored for several years before a transfer, a possible separation of the couple must be considered during this time, which means that the woman would no longer have the option of retransferring of the fertilized cells. To avoid this risk, even in a stable partnership, it is recommended that all oocytes are frozen unfertilised or that splitting is carried out (50% fertilised, 50% unfertilised cryopreserved) to guarantee the woman's independence.

A. Germeyer (✉)
Department of Gynaecological Endocrinology and Fertility Disorders, University Women's Hospital Heidelberg, Heidelberg, Germany
e-mail: Ariane.Germeyer@med.uni-heidelberg.de

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_19

Table 1 Advantages and disadvantages of ovarian stimulation

Advantage	Disadvantage
Established procedure	Time necessary approximately 2 weeks
Well-known chance of conception, dependent on female age and ovarian reserve	Success rate dependent on ovarian reserve (antral follicle count, AMH concentration)
Procedure possible even with low ovarian reserve	Hormone exposure (cave: Hormone dependent tumours)
Also possible with ovarian metastasis	Relatively high cost
	Vaginal sonography and transvaginal follicle aspiration required

Table 2 Average number of oocytes collected from 809 women according to the *Ferti*PROTEKT register [1]

Age (years)	Average number of retrieved oocytes (*n*)
<30	11.7
31–35	12.8
36–40	8.4
>40	4.6

Ovarian stimulation which begins at the time of menstruation is a routine procedure. Measures to minimize the risk of ovarian hyperstimulation syndrome are also clinically established (Table 1).

Stimulation started after menstruation, e.g. in the luteal phase to limit the duration of stimulation to 2 weeks, has already been tested in several clinical studies [2–4]. Double stimulation [5–7] and stimulation immediately after removal of ovarian tissue have also been the subject of several studies [8, 9].

Effectiveness

The number of oocytes collected depends on the age of the patient and the underlying individual ovarian reserve. However, the underlying disease has very little effect on the number of oocytes obtained [10, 11]. According to the *Ferti*PROTEKT register, the average number of oocytes obtained from 809 women was [1] (Table 2):

Exceptions [1] are women who have undergone a surgical intervention on the ovaries before stimulation (see chapter “Ovarian Tumors and Ovarian Cancer”) or with Hodgkin’s lymphoma (see chapter “Hodgkin’s Lymphoma”). Fewer oocytes may be obtained after ovarian stimulation in women with a BRCA mutation, presumably due to a lower ovarian reserve (see chapter “Breast Cancer”).

If vitrification is adequately performed as a freezing technique, the survival and fertilisation rates of cryopreserved oocytes are very good. Numerous studies comparing unfertilised and fertilised cryopreserved oocytes with eggs without cryopreservation showed no relevant differences in pregnancy rates [12–14].

Table 3 Theoretical birth rate per stimulation depending on the age of the woman, calculated based on 125 follicular aspirations [15]

Age (years)	Average number of retrieved oocytes (*n*)	Average number of fertilised oocytes (*n*)	Estimated live birth rate (%)
<26	13.5	8.6	~ 40
26–30	11.3	7.3	~ 35
31–35	11.0	6.1	~ 30
36–40	8.3	5.1	~ 20

The effectiveness of oocyte vitrification has also been confirmed in oncological patients. In 11 women whose oocytes were collected at an average age of 35.6 years (30–41) and thawed and fertilized after the disease, an oocyte survival rate of 92%, a fertilization rate of 77% and an implantation rate of 64% were found. Pregnancy occurred in 7 of the 11 women and 4 (36%) gave birth [15].

Based on the number of oocytes that were removed and successfully fertilized before cytotoxic therapy, a *Ferti*PROTEKT [15] study calculated the theoretical birth rate depending on the age of the woman using 125 follicular aspirations (Table 3):

Therefore, after ovarian stimulation with cryopreservation of unfertilized or fertilized oocytes, the theoretical birth rates mentioned in Table 2 can be assumed if the specified number of oocytes is obtained.

Risks

Ovarian stimulation can lead to side effects from the medication, as well as complications during follicular puncture (www.deutsches-ivf-register.de). Women may experience temporary weight gain, mood swings and a feeling of abdominal pressure due to the increase in size of the ovaries. Clinically relevant bleeding from follicular puncture or inflammation is rare. During stimulation of patients in the *Ferti*PROTEKT network, severe ovarian hyperstimulation syndrome occurred only once in 708 stimulations [4]. Chemotherapy has so far only had to be postponed once by one day.

In addition, Del Pup & Peccatori [16] were unable to demonstrate a worsening of prognosis in hormone receptor-positive breast cancer patients stimulated with letrozole compared to non-stimulated controls.

Practical Approach

General

The standard protocol for stimulation is the antagonist protocol with ovulation induction using a GnRH agonist (GnRHa) (triptorelin 0.2 mg s.c.) to minimize the risk of the ovarian overstimulation syndrome [17]. In practice, a daily gonadotropin

dose which is approximately 50 IU higher than that of stimulation with an intended fresh transfer is often administered to increase the number of oocytes.

Random Start Stimulation

Ovarian stimulation can be started at any time during the menstrual cycle with the exclusive aim of collecting oocytes (without fresh embryo transfer) with the same number of oocytes and the same fertilisation rates [1–3] ("random start stimulation"). Pregnancy rates are equally high compared to standard stimulation protocolls, when stimulation is started in the luteal phase [18] and the malformation rates are unaffected [19]. Identical results have also been demonstrated for egg donors and there is currently no disadvantage from "random start" stimulation [20]. According to previous studies, stimulation takes 1–2 days longer when stimulation is started in the luteal phase than when stimulation begins in the early follicular phase.

Stimulation can be performed as follows, depending on the cycle phase (Fig. 1):

- *Start of stimulation in the early and middle follicular phase:* conventional antagonist protocol with FSH or FSH/LH, addition of an GnRH antagonist (GnRHant) if dominant follicle >13 mm and GnRH agonist triggering with triptorelin 0.2 mg s.c. if 3 follicles ≥17 mm. Stimulation dose approx. 50 IU higher than with an intended fresh transfer.

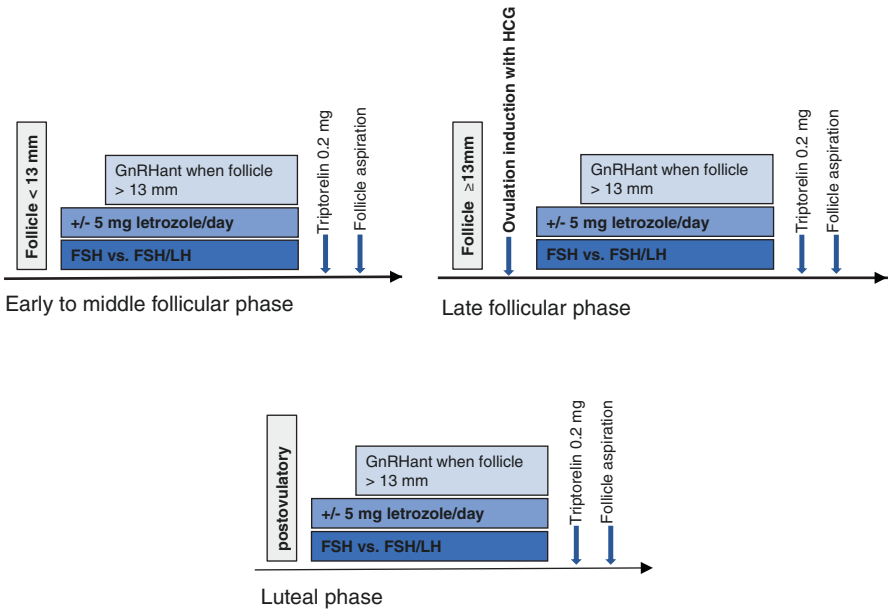


Fig. 1 Ovarian stimulation in different cycle phases

- *Stimulation start in the late follicular phase with a dominant follicle ≥ approx. 13 mm*: ovulation induction with triptorelin 0.2 mg s.c., followed by luteal phase stimulation directly after ovulation.
- *Stimulation start in the luteal phase*: conventional antagonist protocol with FSH or FSH/LH, and GnRH agonist triggering with triptorelin 0.2 mg s.c. Stimulation dose approx. 75 IU higher than with an intended fresh transfer after a stimulation start in the early follicular phase. GnRH antagonist started when new dominant follicle > approx. 13 mm.

Progesterone Primed Ovarian Stimulation

Progesterone-primed ovarian stimulation (PPOS) was derived from luteal phase stimulation, which showed that no LH increase occurs under the influence of progesterone. Since the function of the endometrium does not play a role in fertility preservation (freeze-all strategy), the negative effect of progesterone on the endometrium does not come into effect [21]. Efficient ovulation inhibition, a low rate of side effects and predominantly comparable oocyte counts, fertilisation, implantation and pregnancy rates were demonstrated for 4 and 10 mg medroxyprogesterone acetate (MPA), 10 and 20 mg dydrogesterone and 100 and 200 mg oral micronized progesterone.

The only exception was the latest study by Begueria et al. [22], which showed a lower efficiency of 10 mg MPA compared to the GnRH antagonist protocol in an egg donation programme. Furthermore, in the studies published to date (now >2600 women), there were no increased malformations among the children. In the protocols, the progestins were started on the third day of the cycle at the same time as the gonadotrophins (Fig. 2). It should be noted that an approx. 25 IE higher gonadotrophin dose/dose must be administered to obtain the same number of oocytes and the stimulation takes about 1 day longer (corresponding to luteal phase stimulation). When using MPA and dydrogesterone, the physiological production of progesterone in serum can be demonstrated, whereas this is not the case for oral micronized progesterone. The use of gestagens for ovulation suppression is also conceivable in principle with “random-start” protocols, but their efficiency has yet to be proven.

Double Stimulation

Double stimulation allows an increase in the number of oocytes obtained within approximately 4 weeks [5, 7, 23] (Fig. 3). Most double stimulations are “poor responders”, but they have also been described occasionally in the context of fertility preservation. The similar stimulation protocols all resulted in a higher number of mature oocytes the second stimulation with good developmental quality (summarised in [6, 18]).

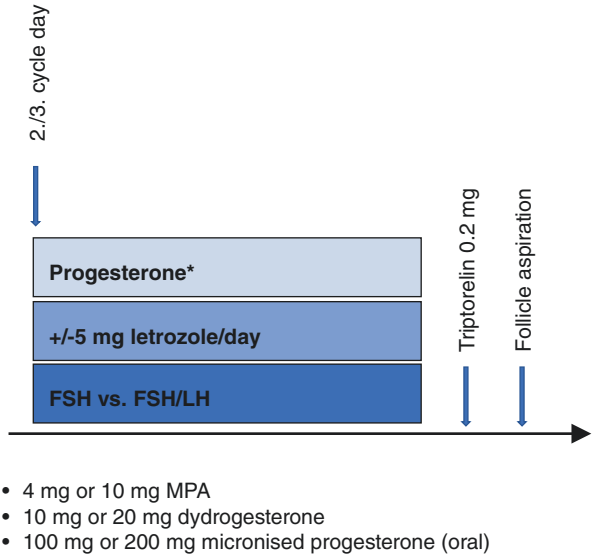


Fig. 2 Progesterone-primed ovarian stimulation (PPOS)

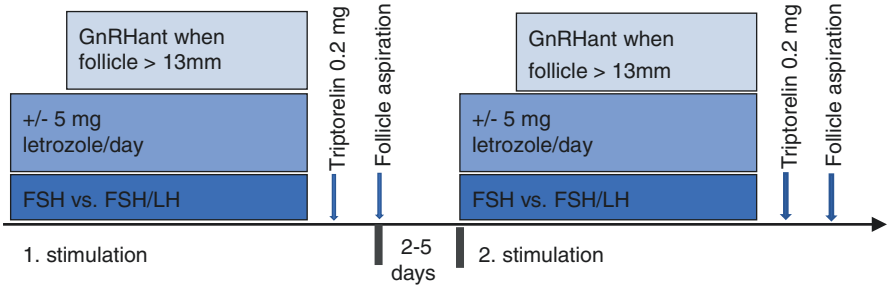


Fig. 3 Double stimulation

With this method, stimulation with a classical antagonist protocol and follicle aspiration is performed first after ovulation induction with a GnRHa. It can be assumed that the first stimulation can also be started in every cycle phase (random start stimulation). A second stimulation is started 2–5 days after follicular puncture according to the luteal phase stimulation described above [23]. A prerequisite is that the ovary does not have too many large follicles (whether the administration of GnRHant for 2 days after follicle puncture accelerates luteolysis has not yet been proven). To rule out premature ovulation, GnRHant are administered additionally as

soon as the new dominant follicle exceeds a size of 13 mm [7] (Fig. 3). Double stimulation takes approximately 30 days.

Reduction of Estradiol Concentration in Hormone Receptor Positive Tumours

Hormone-receptor-positive tumours, in particular hormone receptor-positive breast cancer, represent a special case, since their growth could at least theoretically be increased by supraphysiological estrogen concentrations.

To reduce the increasing estrogen concentrations during ovarian stimulation, the addition of aromatase inhibitors, e.g. letrozole 5 mg (2.5 mg each morning and evening from the first day of stimulation), is recommended for ovarian stimulation in hormone receptor-positive patients [14]. Since letrozole is not approved for use in ovarian stimulation, treatment is off-label. Previous studies have not shown increased malformation rates in children after stimulation with letrozole [24, 25]. The number of oocytes [26] and the pregnancy rates described so far [14, 27] are also unaffected by the addition of letrozole. A more recent study of egg cell quality after letrozole stimulation and an ovulation trigger with GnRHa showed good egg cell quality based on an analysis of gene expression of granulosa cells and local estrogen concentration in the follicular fluid [28]. Oktay et al. [29] recommended that ovulation should only be triggered when the follicle size reaches 20 mm.

It should be noted that stimulation of a hormone receptor-positive patient should only take place after consultation with the responsible oncologists.

Combination of Ovarian Stimulation with the Removal of Ovarian Tissue

Ovarian stimulation can be combined with cryopreservation of ovarian tissue to increase the chance of success in treatments with high gonadotoxicity (Fig. 4) [7, 8]. Ovarian stimulation begins approximately 2 days after the laparoscopic removal of 50% of an ovary. In addition, a GnRHa depot injection could be given for fertility preservation on the day of follicular puncture. According to the studies carried out so far, there is no increased risk of complications. The number of oocytes obtained is not reduced after the removal of ovarian tissue. The time required for the combination of both therapies is approximately 2.5 weeks.

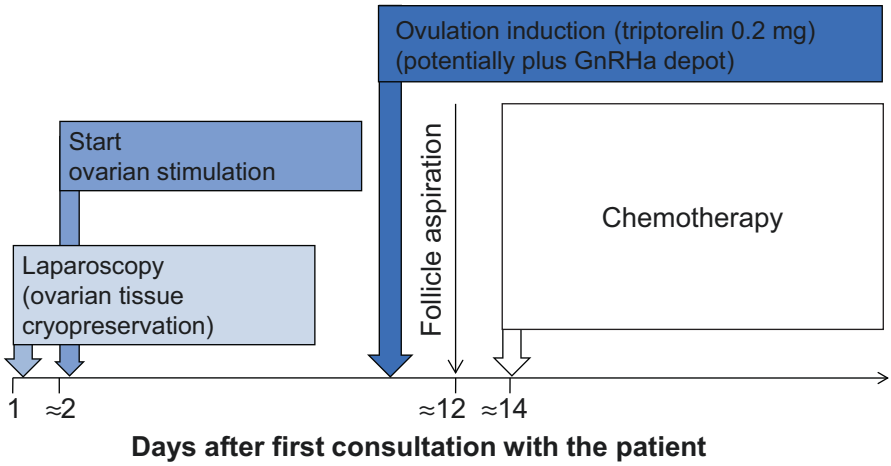


Fig. 4 Combination of ovarian stimulation with the removal of ovarian tissue and the administration of a GnRHa

References

1. von Wolff M, Dittrich R, Liebenthron J, Nawroth F, Schüring AN, Bruckner T, Germeyer A. Fertility-preservation counselling and treatment for medical reasons: data from a multinational network of over 5.000 women. Reprod Biomed Online. 2015;31:605–12.
2. von Wolff M, Thaler CJ, Frambach T, Zeeb C, Lawrenz B, Popovici RM, Strowitzki T. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. Fertil Steril. 2009;92:1360–5.
3. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. Fertil Steril. 2013;100:1673–80.
4. von Wolff M, Capp E, Jauckus J, Strowitzki T, Germeyer A. Timing of ovarian stimulation in patients prior to gonadotoxic therapy – an analysis of 684 stimulations. Eur J Obstet Gynecol Reprod Biol. 2016;199:146–9.
5. Moffat R, Pirtea P, Gayet V, Wolf JP, Chapron C, de Ziegler D. Dual ovarian stimulation is a new viable option for enhancing the oocyte yield when the time for assisted reproductive technology is limited. Reprod Biomed Online. 2014;29:659–61.
6. Zhang J. Luteal phase ovarian stimulation following oocyte retrieval: is it helpful for poor responders? Reprod Biol Endocrinol. 2015;13:76.
7. Turan V, Bedoschi G, Moy F, Oktay K. Safety and feasibility of performing two consecutive ovarian stimulation cycles with the use of letrozole-gonadotropin protocol for fertility preservation in breast cancer patients. Fertil Steril. 2013;100:1681–5.
8. Huober-Zeeb C, Lawrenz B, Popovici RM, Strowitzki T, Germeyer A, Stute P, von Wolff M. Improving fertility preservation in cancer: ovarian tissue cryobanking followed by ovarian stimulation can be efficiently combined. Fertil Steril. 2011;95:342–4.
9. Dolmans MM, Marotta ML, Pirard C, Donnez J, Donnez O. Ovarian tissue cryopreservation followed by controlled ovarian stimulation and pick-up of mature oocytes does not impair the number or quality of retrieved oocytes. J Ovarian Res. 2014;7:80.
10. Turan V, Quinn MM, Dayioglu N, Rosen MP, Oktay K. The impact of malignancy on response to ovarian stimulation for fertility preservation: a meta-analysis. Fertil Steril. 2018;110:1347–55.

11. von Wolff M, Bruckner T, Strowitzki T, Germeyer A. Fertility preservation: ovarian response to freeze oocytes is not affected by different malignant diseases - an analysis of 992 stimulations. J Assist Reprod Genet. 2018;35:1713–9.
12. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, Colamaria S, Sapienza F, Ubaldi F. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod. 2010;25:66–73.
13. Martinez M, Rabadan S, Domingo J, Cobo A, Pellicer A, Garcia-Velasco JA. Obstetric outcome after oocyte vitrification and warming for fertility preservation in women with cancer. Reprod Biomed Online. 2014;29:722–8.
14. Oktay K, Turan V, Bedoschi G, Pacheco FS, Moy F. Fertility preservation success subsequent to concurrent aromatase inhibitor treatment and ovarian stimu- lation in women with breast cancer. J Clin Oncol. 2015;33:2424–9.
15. Lawrenz B, Jauckus J, Kupka M, Strowitzki T, von Wolff M. Efficacy and safety of ovarian stimulation before chemotherapy in 205 cases. Fertil Steril. 2010;94:2871–3.
16. Del Pup L, Peccatori FA. Is ovulation induction with letrozole in breast cancer patients still safe even if it could increase progesterone levels? Eur Rev Med Pharmacol Sci. 2018;22:246–9.
17. Youssef MA, Abdelmoty HI, Ahmed MA, Elmohamady M. GnRH agonist for final oocyte maturation in GnRH antagonist co-treated IVF/ICSI treatment cycles: systematic review and meta-analysis. J Adv Res. 2015;6:341–9.
18. Kuang Y, Hong Q, Chen Q, Lyu Q, Ai A, Fu Y, Shoham Z. Luteal-phase ovarian stimulation is feasible for producing competent oocytes in women undergoing in vitro fertilization/intracytoplasmic sperm injection treatment, with optimal pregnancy outcomes in frozen-thawed embryo transfer cycles. Fertil Steril. 2014;101:105–11.
19. Chen H, Wang Y, Lyu Q, Ai A, Fu Y, Tian H, Cai R, Hong Q, Chen Q, Shoham Z, Kuang Y. Comparison of live-birth defects after luteal-phase ovarian stimulation vs. conventional ovarian stimulation for in vitro fertilization and vitrified embryo transfer cycles. Fertil Steril. 2015;103:1194–201.
20. Martínez F, Clua E, Devesa M, Rodríguez I, Arroyo G, González C, Solé M, Tur R, Coroleu B, Barri PN. Comparison of starting ovarian stimulation on day 2 versus day 15 of the menstrual cycle in the same oocyte donor and pregnancy rates among the corresponding recipients of vitrified oocytes. Fertil Steril. 2014;102:1307–11.
21. La Marca A, Capuzzo M. Use of progestins to inhibit spontaneous ovulation during ovarian stimulation: the beginning of a new era? Reprod Biomed Online. 2019;39:321–31.
22. Begueria R, Garcia D, Vassena R, Rodriguez A. Medroxyprogesterone acetate versus ganirelix in oocyte donation: a randomized controlled trial. Hum Reprod. 2019;34:872–80.
23. Sighinolfi G, Sunkara SK, La Marca A. New strategies of ovarian stimulation based on the concept of ovarian follicular waves: from conventional to random and double stimulation. Reprod Biomed Online. 2018;37:489–97.
24. Sharma S, Ghosh S, Singh S, Chakravarty A, Ganesh A, Rajani S, Chakravarty BN. Congenital malformations among babies born following letrozole or clomiphene for infertility treatment. PLoS One. 2014;9:e108219.
25. Kim J, Turan V, Oktay K. Long-term safety of letrozole and gonadotropin stimulation for fertility preservation in women with breast cancer. J Clin Endocrinol Metab. 2016;101:1364–71.
26. Pereira N, Hancock K, Cordeiro CN, Lekovich JP, Schattman GL, Rosenwaks Z. Comparison of ovarian stimulation response in patients with breast cancer undergoing ovarian stimulation with letrozole and gonadotropins to patients undergoing ovarian stimulation with gonadotropins alone for elective cryopreservation of oocytes. Gynecol Endocrinol. 2016;32:823–6.
27. Haas J, Bassil R, Meriano J, Samara N, Barzilay E, Gonen N, Casper RF. Does daily co-administration of letrozole and gonadotropins during ovarian stimulation improve IVF outcome? Reprod Biol Endocrinol. 2017;15:70.
28. Goldrat O, Van Den Steen G, Gonzalez-Merino E, Dechène J, Gervy C, Delbaere A, Devreker F, De Maertelaer V, Demeestere I. Letrozole-associated controlled ovarian hyperstimulation

in breast cancer patients versus conventional controlled ovarian hyperstimulation in infertile patients: assessment of oocyte quality related biomarkers. Reprod Biol Endocrinol. 2019;17:3.
29. Oktay K, Hourvitz A, Sahin G, Oktem O, Safro B, Cil A, Bang H. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J Clin Endocrinol Metab. 2006;91:3885–90.

Cryopreservation of Unfertilized and Fertilized Oocytes

Jana Liebenthron and Jens Hirchenhain

Introduction

Cryopreservation of unfertilized oocytes or fertilized oocytes after in vitro fertilization (IVF) and/or intracytoplasmic sperm injection (ICSI) (at the pronucleus or embryonic stage) is an established and standardized reproductive medical technique that can be reliably used in patients prior to fertility-damaging or -reducing treatment [1, 2]. It should be taken into account here that a post-pubertal patient has a window of about 2 weeks until the start of her treatment, and, as a further prerequisite, she must have an expected good ovarian response to the necessary hormone stimulation (see chapter "Ovarian Stimulation").

Ultra-Fast Freezing (Vitrification)

The absence of intracellular ice crystal formation is made possible by converting the oocyte from a liquid to an amorphous, glassy state. To achieve this glass-like solidification within the oocyte and extracellularly, and to minimize toxic and osmotic injuries, extremely high cooling rates, highly concentrated commercial cryoprotectants (mixture of permeable and non-permeable cryopreservatives, which also

J. Liebenthron (✉)
UniCareD, University Cryobank for Assisted Reproductive Medicine and Fertility Protection at UniKiD Duesseldorf, University Hospital Duesseldorf, Duesseldorf, Germany
e-mail: Jana.Liebenthron@unicared.de

J. Hirchenhain
UniKiD, University Centre for Assisted Reproductive Medicine, University Hospital Duesseldorf, Duesseldorf, Germany
e-mail: hirchenhain@unikid.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_20

reduce the specific toxicity of each component) and minimal volumes (in the nanolitre range) must be used [3].

In vitrification, a distinction is made between two user systems that refer to the cell carrier: an open and a closed system. The open system establishes direct contact between the cell to be frozen and liquid nitrogen which allows very high cooling rates to be achieved. A closed system generates slightly lower cooling rates compared to the open system which may result in the repeatedly discussed lower success rates but avoids the theoretical risk of contamination and infection of the germ cell.

Oocyte Retrieval

Ovarian stimulation is required to cryopreserve a sufficient number of oocytes, which, thanks to modern stimulation protocols, can now start at any point in the cycle ("random start stimulation") [2, 4–6] (see chapter "Ovarian Stimulation to Collect Oocytes"). On average, about 13 oocytes can be obtained before cytotoxic treatment [4]. However, the number of oocytes obtained decreases significantly at the age of >35 years. A detailed description of the stimulation techniques, their effectiveness, risks and costs can be found in Chapter "Ovarian Stimulation to Collect Oocytes".

Cryopreservation of Unfertilized Oocytes

Freezing the particularly cryosensitive unfertilized oocytes was the greatest challenge in the cryopreservation process of female gonadal cells. This is due to their low membrane permeability, their size (130 µm), their high water content (danger of intracellular ice crystal formation if handled incorrectly) and the presence of the still intact spindle apparatus due to unfinished meiosis [7]. According to the current ASCO (American Society of Clinical Oncology) guidelines [8], ESHRE/ASRM (European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine) [9], national and international recommendations [1, 10] as well as the AWMF S2k guideline from the German, Austrian and Swiss Societies of Gynaecology and Obstetrics (DGGG, OEGGG, SGGG) on fertility preservation in oncological disease [11], vitrification is considered the standard method for cryopreservation of unfertilized oocytes. This is because unfertilized oocytes that had undergone slow freezing could only be very poorly fertilized after thawing and had generally poorer survival rates, and fertility preservation by cryopreservation of unfertilized oocytes only really made sense after the introduction of vitrification [7]. The success rates published in the German, Austrian and Swiss AWMF guideline [11] after vitrification are shown in Table 1 and can be traced back to papers published at that time [12, 13].

Table 1 Success rates after vitrification of unfertilized oocytes [11]

Survival rate per unfertilized oocyte after cryopreservation/thawing (%)	80–90
Fertilization rate per unfertilized oocyte after cryopreservation/thawing (%)	76–83
Clinical pregnancy rate (%)	44.9
Malformation rate (%)	1.3

Table 2 Age-related cumulative results of patients with oocyte vitrification before oncological treatment [15]

Age-groups	≤35 years	>35 years
Number of patients (*n*)	42	38
Number of vitrification/thawing cycles (*n*)	42	39
Mean age (years ± SD)	31.6 ± 2.1	38.0 ± 2.1
Oocyte survival rate (%)	81.2	82.7
Clinical pregnancies (*n*)/thawing cycles (*n*) (%)	18 /42 (42.8)	15/39 (38.5)
Ongoing pregnancies (*n*)/thawing cycles (*n*) (%)	15/42 (35.7)	10/39 (25.6)
Live births (*n*)/patients (*n*) (%)	16/38 (42.1)	9/33 (29.0)

If more recent figures from Cobo et al. [14, 15] are considered, further improved survival rates after cryopreservation/thawing of 80–95%, average clinical pregnancy rates after ICSI per embryo transfer of 30% (10–59%) and an average cumulative live birth rate of 33% (6-62%) are seen for vitrification. The success rates (especially the live birth rate per oocyte) correlate strongly with the age of the patients at collection/vitrification and the number of oocytes collected and matured [14–16]. Patients ≤36 years of age achieve better results than older patients [14–19], especially since with advanced age not only the quality of the oocytes decreases but also the number of oocytes to be collected and matured due to the age-related reduction in the ovarian reserve.

A recent study by Cobo et al. [15] examined the largest series of vitrified oocytes from a patient group undergoing fertility preservation prior to oncological treatment. It therefore provided data on the influence fertility preservation indication on the results by comparing IVF data, oocyte survival, clinical outcomes and live birth rates (Table 2).

The majority of patients in this group were treated for breast cancer (64.6%), followed by women with Hodgkin's (11.6%) or non-Hodgkin's lymphoma (5.2%). A GnRH antagonist protocol plus letrozole for stimulation was used in 72.8%.

Numerous publications have been published which, using retrospectively collected data, have calculated the birth rate depending on the number of mature oocytes and on the woman's age [16, 20]. Furthermore, Doyle et al. [16] performed a calculation using fresh and cryopreserved oocytes from women with sterility and designed useful graphics for everyday practice, for a better illustration for patients and users. They indicated in detail age-associated oocyte to child efficiencies, ranging from 8.7% for women aged <30 years to 1.1% for women aged 43–44 years (Table 3), and an overall oocyte to child efficiency of 6.7%.

Table 3 Age-related calculation of the live birth rate per thawed oocyte [16]

Age groups	Probability of children per thawed oocyte (%)
<30 years	8.67
30–34 years	8.20
35–37 years	7.33
38–40 years	4.47
41–42 years	2.49
43–44 years	1.06

Therefore, attempting fertility preservation after the age of 40 years is unlikely to be successful. The relative stability of fertility potential in the healthy population through the early to mid-30s suggests this would be a reasonable span in which to consider oocyte cryopreservation [16].

Irrespective of this, the success rates strictly correlate with the user, that is good success rates after vitrification can only be achieved with practiced and safe handling of the cryopreservation technique [12, 19]. Improper or uncertain implementation in the freezing and/or thawing process as well as non-compliance with the strict time requirements during the individual incubation steps with highly concentrated cryoprotective agents lead to irreversible damage to the germ cells, which is clearly reflected in the survival, fertilization, development, clinical pregnancy and live birth rates. However, it is possible that automation of the vitrification process in the future will stabilize the high success rates through standardized work processes in a controlled environment that are independent of the skill of the user.

Furthermore, the currently available evidence suggests that cryopreservation of unfertilized oocytes, compared to fresh cycles [7], is not associated with increased obstetric and perinatal complications—although large long-term follow-up studies are still lacking.

Nevertheless, the potential benefits of cryopreservation of oocytes must be weighed against the risks and costs. Stimulation, aspiration and cryopreservation of oocytes in patients with an unfavourable prognosis, and/or from a psychological point of view if there is a great fear of infertility despite only very weak chemotherapy, must be avoided. The benefit is particularly defined by the later use of the oocytes. The later use rate is low according to previous studies (Table 4).

Cryopreservation of Fertilized Oocytes

Cryopreservation of Pronuclear Stages

In contrast to the cryopreservation of unfertilized oocytes, the cryopreservation of fertilized oocytes with two pronuclei (pronucleus stage) is a method that has a long safety record and is an essential component of assisted reproductive medicine techniques.

Table 4 Retrieval rate of cryopreserved oocytes before gonadotoxic therapies and outcome [15, 21]

	Cobo et al. 2018 [15]	Diaz-Garcia et al. 2018 [21]
Number of patients with cryopreserved oocytes, n	1073	1024
Patients who have retrieved their oocytes, *n* (%)	80/1073 (7.4)	49/1024 (4.8)
Transfers/warming cycle (%)	58/80 (72.5)	n.a.
No. of embryos transferred/cycle	1.4	1.4
Implantation rate (%)	32.5	n.a.
Clinical pregnancies/transfer (%)	24/58 (41.4)	20/49 (40.8)
Ongoing pregnancies/transfer (%)	18/58 (31.0)	16/49 (32.6)
Live births (%)	18/58 (31.0)	16/49 (32.6)

According to current figures from the DIR (German IVF Register) Annual report 2019 [22], the clinical pregnancy rate per transfer of around two thawed procucleus stages is 28% on average. The absence of the spindle apparatus after completion of meiosis makes the oocyte much easier to treat in the laboratory [23]. Cryopreservation can be carried out using the slow-freezing method as well as by vitrification. However, vitrification is also becoming the standard method here. If cryopreservation of fertilized oocytes is planned, the patient should be in a stable relationship. To preserve the woman's independence, splitting (50% fertilized, 50% unfertilized cryopreserved) is recommended, even if she is in a permanent relationship. It may even be necessary to consider cryopreservation of all oocytes in an unfertilized state.

Cryopreservation of Embryos

Cryopreservation of embryos, preferably in the blastocyst stage, is also an established method in most countries and is also carried out by vitrification. The survival rate after thawing is approximately 97%. The clinical pregnancy rate per thawing cycle is approximately 35–41% [24], almost twice as high as for the cryopreservation of pronucleus stages. The reason for this is that only about 50% of pronucleus stages reach the blastocyst stage—the effectiveness between pronucleus and blastocyst stage is therefore hardly comparable.

Which stage of development (oocyte, pronucleus stage or embryo) is ultimately preferred for cryopreservation depends not only on the country's legal requirements but also on the personal interests of the patient.

Conclusion

The introduction of new stimulation protocols and the establishment of vitrification make it possible to obtain oocytes within about 2 weeks, which can be cryopreserved either unfertilized or fertilized in the pronucleus stage or as blastocysts.

The German, Austria and Swiss AWMF guideline [11] defined the following consensus-based statements and recommendations in the field of cryopreservation of unfertilized and/or fertilized oocytes for fertility preservation, each with very strong consensus strengths of +++:

- "The cryopreservation of fertilized and unfertilized oocytes is an established reproductive medical technique that can be used prior to gonadotoxic treatment".
- "The cryopreservation of unfertilized oocytes does not show an increased rate of malformations or developmental deficits in children compared to the cryopreservation of fertilized oocytes".
- "Freezing of unfertilized oocytes should also be offered, even in an existing relationship".

References

1. Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim HA Jr, Peccatori FA, et al. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med. 2016;14:1. https://doi.org/10.1186/s12916-015-0545-7.
2. von Wolff M, Dittrich R, Liebenthron J, Nawroth F, Schüring A, Bruckner T, et al. Fertility-preservation counselling and treatment for medical reasons: data from a multinational network of over 5000 women. Reprod Biomed Online. 2015;31(5):605–12. https://doi.org/10.1016/j.rbmo.2015.07.013. Epub 2015 Aug 11
3. Nawroth F. Aktuell diskutiert: Reproduktionsmedizin. Vitrifikation Geburtshilfe Frauenheilkd. 2015;75(09):883–6. https://doi.org/10.1055/s-0035-1557814.
4. Polat M, Bozdag G, Yarali H. Best protocol for controlled ovarian hyperstimulation in assisted reproductive technologies: fact or opinion? Semin Reprod Med. 2014;32(4):262–71. https://doi.org/10.1055/s-0034-1375178. Epub 2014 Jun 11
5. von Wolff M, Thaler CJ, Frambach T, Zeeb C, Lawrenz B, Popovici RM, et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. Fertil Steril. 2009;92(4):1360–5. https://doi.org/10.1016/j.fertnstert.2008.08.011. Epub 2008 Oct 18
6. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. Fertil Steril. 2013;100(6):1673–80. https://doi.org/10.1016/j.fertnstert.2013.07.1992. Epub 2013 Aug 26
7. Iussig B, Maggiulli R, Fabozzi G, Bertelle S, Vaiarelli A, Cimadomo D, et al. A brief history of oocyte cryopreservation: arguments and facts. Acta Obstet Gynecol Scand. 2019;98(5):550–8. https://doi.org/10.1111/aogs.13569. Epub 2019 Mar 25
8. Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, et al. Fertility preservation in patients with cancer: ASCO clinical practice guideline update. J Clin Oncol. 2018;36(19):1994–2001. https://doi.org/10.1200/JCO.2018.78.1914. Epub 2018 Apr 5
9. Martinez F, International Society for Fertility Preservation Working Group. Update on fertility preservation from the Barcelona International Society for Fertility Preservation-ESHRE-ASRM 2015 expert meeting: indications, results and future perspectives. Fertil Steril. 2017;108(3):407–415.e11. https://doi.org/10.1016/j.fertnstert.2017.05.024. Epub 2017 Jul 21
10. von Wolff M, Germeyer A, Liebenthron J, Korell M, Nawroth F. Practical recommendations for fertility preservation in women by the FertiPROTEKT network. Part II: fertility preservation techniques. Arch Gynecol Obstet. 2018;297(1):257–67. https://doi.org/10.1007/s00404-017-4595-2. Epub 2017 Nov 27

11. Deutsche Gesellschaft für Gynäkologie und Geburtshilfe (DGGG), Deutsche Gesellschaft für Reproduktionsmedizin (DGRM), Deutsche Gesellschaft für Urologie (DGU). Leitlinie: Fertilitätserhaltung bei onkologischen Therapien. Level S2k, AWMF Register Nr. 015/082, November 2017. http://www.awmf.org/leitlinien/detail/ll/015-082.html.
12. LPE S, Porcu E, Patrizio P, Vigiliano V, de Luca R, d'Aloja P, Spoletini R, Scaravelli G. Human oocyte cryopreservation with slow freezing versus vitrification. Results from the National Italian Registry data, 2007–2011. Fertil Steril. 2014;102(1):90–95.e2. https://doi.org/10.1016/j.fertnstert.2014.03.052. Epub 2014 Apr 29
13. Glujovsky D, Riestra B, Sueldo C, Fiszbajn G, Repping S, Nodar F, et al. Vitrification versus slow freezing for women undergoing oocyte cryopreservation. Cochrane Database Syst Rev. 2014;9:CD010047. https://doi.org/10.1002/14651858.CD010047.pub2.
14. Cobo A, García-Velasco JA, Coello A, Domingo J, Pellicer A, Remohí J. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril. 2016;105:755–64.e8. https://doi.org/10.1016/j.fertnstert.2015.11.027. Epub 2015 Dec 10
15. Cobo A, García-Velasco JA, Domingo J, Pellicer A, Remohí J. Elective and onco-fertility preservation: factors related to IVF outcomes. Hum Reprod. 2018;33(12):2222–31. https://doi.org/10.1093/humrep/dey321.
16. Doyle JO, Richter KS, Lim J, Stillman RJ, Graham JR, Tucker MJ. Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with predicted birth probabilities for fertility preservation according to number of cryopreserved oocytes and age at retrieval. Fertil Steril. 2016;105(2):459–66.e2. https://doi.org/10.1016/j.fertnstert.2015.10.026. Epub 2015 Nov 18
17. Coello A, Pellicer A, Cobo A. Vitrification of human oocytes. Minerva Ginecol. 2018;70(4):415–23. https://doi.org/10.23736/S0026-4784.18.04218-1. Epub 2018 Mar 8. Review
18. Cil AP, Bang H, Oktay K. Age-specific probability of live birth with oocyte cryopreservation: an individual patient data meta-analysis. Fertil Steril. 2013;100:492–9.e3. https://doi.org/10.1016/j.fertnstert.2013.04.023. Epub 2013 May 24
19. Solé M, Santaló J, Boada M, Clua E, Rodríguez I, Martínez F, et al. How does vitrification affect oocyte viability in oocyte donation cycles? A prospective study to compare outcomes achieved with fresh versus vitrified sibling oocytes. Hum Reprod. 2013;28(8):2087–92. https://doi.org/10.1093/humrep/det242. Epub 2013 Jun 5
20. Goldman RH, Racowsky C, Farland LV, Munné S, Ribustello L, Fox JH. Predicting the likelihood of live birth for elective oocyte cryopreservation: a counseling tool for physicians and patients. Hum Reprod. 2017;32(4):853–9. https://doi.org/10.1093/humrep/dex008.
21. Diaz-Garcia C, Domingo J, Garcia-Velasco JA, Herraiz S, Mirabet V, Iniesta I, et al. Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for adult women undergoing gonadotoxic treatments: a prospective cohort study. Fertil Steril. 2018;109(3):478–485.e2. https://doi.org/10.1016/j.fertnstert.2017.11.018. Epub 2018 Feb 7
22. Jahrbuch 2017 des Deutschen IVF-Registers. J Reproduktionsmed Endokrinol 2019;16: modifizierter Nachdruck aus Nummer 6: 279-315// ISSN 1810-2107, Sonderheft 1 2019. https://www.deutsches-ivf-register.de/perch/resources/dir-jahrbuch-2018-deutsch-4.pdf.
23. Nawroth F. Social freezing. Berlin: Springer Verlag; 2015ISBN 978-3-658-09892-6. https://doi.org/10.1007/978-3-658-09892-6.
24. Cobo A, de los Santos MJ, Castellò D, Gámiz P, Campos P, Remohí J. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. Fertil Steril. 2012;98(5):1138–46.e1. https://doi.org/10.1016/j.fertnstert.2012.07.1107. Epub 2012 Aug 3

Removal of Ovarian Tissue

Michael von Wolff and Jana Liebenthron

Introduction

The removal and cryopreservation of ovarian tissue for later transplantation in patients with therapy-induced premature ovarian insufficiency who wish to have children is an established and standardized reproductive medical technique for post-pubertal patients, and more than 130 live births using this method were documented worldwide in 2017 [1]. There are now over 170 known births. The method is an essential pillar in the field of fertility preservation with many advantages but also some disadvantages (Table 1). Since the ovarian tissue transplants used later allow a temporary restitution of endogenous hormone production, a spontaneous pregnancy is possible. Menopausal symptoms are also reduced. A major advantage is the short period of time required before removal, as no preparation of the patient is necessary, and the tissue can be removed at any time during the menstrual cycle.

Pre-pubertal girls and young women also benefit from cryopreservation of ovarian tissue, and 10 births after cryopreservation of ovarian tissue before the age of 20 years have been reported [2]. In individual cases, puberty was induced with transplanted tissue [3].

The following principle applies when removing ovarian tissue: as much as necessary, as little as possible. Therefore, only half an ovary is removed and cryopreserved in most centres, since it has been proven that pregnancies can be generated

M. von Wolff (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

J. Liebenthron
UniCareD, University Cryobank for Assisted Reproductive Medicine and Fertility Protection at UniKiD Duesseldorf, University Hospital Duesseldorf, Duesseldorf, Germany
e-mail: Jana.Liebenthron@unicared.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_21

Table 1 Possible advantages and disadvantages of removal and cryopreservation of ovarian tissue

Advantages of removal and cryopreserving ovarian tissue	Disadvantages of removal and cryopreserving ovarian tissue
Time required approximately ½–1 week, removal possible at any point in the cycle	Invasive procedure performed under general anaesthesia with increased risks in patients with immunosuppression and blood coagulation disorders
Removal and cryopreservation of ovarian tissue: established procedure	Chances of success depend on the available ovarian reserve, upper age limit approx. 35–38 years
Removal and cryopreservation costs are lower than ovarian stimulation/ cryopreservation of oocytes	Possibly tumour cells in ovarian tissue with the risk of recurrence
Transvaginal ultrasound is not required for the removal and cryopreservation of ovarian tissue	Experimental in diseases with malignancies of the haematopoietic system and in diseases with a high risk of ovarian metastasis
Removal and cryopreservation of ovarian tissue can also be carried out in children	Removal of a whole ovary is necessary in children due to the small size of the ovaries
Fertility preservation method with high potential for development	

even with a smaller amount of tissue without significantly reducing the woman's chances of later spontaneous conception (without transplantation).

Risks of Transplanting Ovarian Tumour Cells

In principle, there is a risk of preserving malignant cells when cryopreserving ovarian tissue and of transferring it when the tissue is transplanted. Because of this, a small piece of tissue is taken for histopathological examination to exclude metastases.

Regardless of these histological and further examinations, the patient should be informed about the risks of ovarian contamination with malignant cells.

Risk categories for ovarian contamination with malignant cells have been defined based on previous publications taking into account the specific tumour biology. Table 2 shows the risk categorization according to the German–Austrian–Swiss AWMF-S2k guidelines [4] and Dolmans & Masciangelo 2018 [5].

Cryopreservation with Malignant Diseases of the Haematopoietic System

Malignant diseases of the haematopoietic system (leukaemia, etc.) pose a problem, as GnRH agonists (GnRHa) are not effective enough in stem cell transplantations and as the window of time available is too short for ovarian stimulation. Therefore, there is usually only the option of experimentally preserving ovarian tissue in addition to GnRHa for bleeding prevention.

Table 2 Risk of ovarian metastasis with different types of tumours [4, 5]

High risk	Moderate risk	Low risk
Leukaemia	Stage IV breast cancer, especially lobular subtypes	Stage I–III breast cancer, especially ductal subtypes
Neuroblastoma	Particularly advanced colorectal cancers	Squamous cell carcinoma of the cervix
Burkitt's lymphoma	Stomach cancer	Hodgkin's lymphoma
Ovarian cancer	Adenocarcinoma of the cervix	Rhabdomyosarcoma
	Non-Hodgkin's lymphoma	Soft tissue sarcomas
	Ewing's sarcoma	
	Borderline ovarian tumours	

Ovarian tissue can be removed either before chemotherapy or in the window of time between induction and bone marrow ablative chemotherapy. Removal before chemotherapy is useful in young women in the expectation that techniques will have been developed by the time the tissue is needed (see chapter "Further Fertility Preservation Techniques"), which will allow risk-free use. Alternatively, the tissue can be removed after induction chemotherapy, since it has been shown that the function of the tissue is not significantly reduced by mild chemotherapy (see section "Cryopreservation of Ovarian Tissue after Starting Chemotherapy"). However, even after induction chemotherapy, there is a risk that the tissue will continue to contain malignant cells.

Shapira et al. [6] described a birth for the first time after transplantation of ovarian tissue taken from a leukaemia patient. After extensive molecular biological tests, the presence of malignant cells in the ovarian tissue could be excluded with a high degree of probability. A relapse of leukaemia did not occur after transplantation.

However, Gook et al. [7] showed in mouse experiments that (1) malignant cells are almost always found in the ovarian tissue of leukaemia patients, that (2) biopsy results cannot be applied to the tissue to be transplanted and that (3) the transfer of malignant cells can lead to leukaemia.

Ovarian tissue transplantation in patients with leukaemia and other malignant diseases of the haematopoietic system is therefore not currently recommended. Ovarian tissue should only be cryopreserved if the risk of intra-ovarian malignant cells is assessed as very low after pre-treatment or if the patient is very young, and it can therefore be speculated that techniques to avoid spread of tumor cells by transplantation will have been developed by the time the tissue is needed.

Cryopreservation of Ovarian Tissue After Starting Chemotherapy

Sometimes chemotherapy must be started urgently before a fertility preservation measure can be started. Under certain circumstances, the decision to carry out a fertility preservation measure may also only be made after the start of

chemotherapy. The reason may be a change in the patient's opinion over time or that a milder chemotherapy was initially started without freezing of oocytes or ovarian tissue.

In these cases, it is not possible to preserve oocytes, especially when alkylating substances are used. Cryopreservation of oocytes can be performed 3 months after completion of chemotherapy at the earliest, the period of maturation from primary to tertiary follicles. However, cryopreservation of ovarian tissue is possible, as undamaged primordial follicles can mature after transplantation of the tissue.

Poirot et al. [8] transplanted ovarian tissue from 22 women who had received chemotherapy before tissue removal. Alkylating agents, among other things, were administered to 20 women. Most of these women had been diagnosed with lymphoma. After initial chemotherapy, for example according to the ABVD scheme, stem cell transplantation with prior cryopreservation of ovarian tissue was performed. After transplantation of ovarian tissue, only the duration of tissue activity was significantly reduced compared to women with cryopreservation without prior chemotherapy; all other parameters, including pregnancy rates, did not differ.

Meirow et al. [9] published a study of 10 women in whom ovarian tissue had also been removed after rather mild initial chemotherapy and later transplanted. In this study, the pregnancy rates did not differ from a control group without prior chemotherapy.

These data show that ovarian tissue can also be cryopreserved after rather mild gonadotoxic chemotherapy.

Effectiveness and Risks

The removal and cryopreservation of ovarian tissue is now an established technique. Tissue removal is simple, quick to perform and therefore very effective.

The risks are low and are only increased in some oncological diseases due to a generally increased risk of infection and bleeding (e.g. in leukaemia).

According to the *Ferti*PROTEKT register, one complication requiring surgical revision can be expected in 500 laparoscopies when ovarian tissue is removed [10]. However, in the register, the proportion of tissue removals in children and women with an increased surgical risk and in diseases with a functional impairment of the immune system and blood clotting is relatively low [11], and a calculation in these risk groups is not possible.

The potential benefits of tissue removal and cryopreservation must be weighed against the risks and costs. Removal and cryopreservation in the event of a poor prognosis and/or for a psychological indication (e.g. great fear of infertility despite chemotherapy that is not or is only slightly gonadotoxic) must be avoided.

Benefit is particularly defined by the subsequent use of the tissue for transplantation. According to previous studies, the usage rate is very low (Table 3), although it can be assumed that it will increase over the coming years. Since the tissue is often only retrieved after several years of storage, the low use rate nevertheless shows that

Table 3 Tissue retrieval rate and frequency of transplants per cryopreservation

	Total number of cryopreservations *n*	Total transplanted patients *n*	Transplanted patients/total number of cryopreservations *n* (%)	Patients with at least one birth/total number of cryopreservations *n* (%)
Van der Ven et al. 2016 (*Ferti*PROTEKT) [12]	2500	49	49/2500 (1.9%)	15/2500 (0.6%)
Jadoul et al. 2017 [13]	545	21	21/545 (3.9%)	7/545 (1.3%)
Diaz-Garcia et al. 2018 [14]	800	44	44/800 (5.5%)	8/800 (1.0%)
Total	**3845**	**114**	**114/3845 (2.9%)**	**30/3845 (0.8%)**

Table 4 Factors determining the preference for cryopreservation of ovarian tissue or oocytes

Factors	Ovarian tissue preferred	Oocytes preferred
Woman's age	Pre/peripubertal age	Age > ca. 35 years
Woman's health status	Major medical risks with ovarian stimulation	Major medical risks with intubation anaesthesia
Time needed	Available timeframe < 2 weeks	Available timeframe ≥ 2 weeks
Ovarian metastasis	Low risk of intraovarian malignant cells	High risk of intraovarian malignant cells
Chemotherapy	Chemotherapy already started	
Radiotherapy		Pelvic radiotherapy (if uterus is not exposed)
Vaginal access	Virgo intacta	

the indication for the removal and cryopreservation of ovarian tissue, as well as that of oocytes (see chapter "Cryopreservation of Unfertilized and Fertilized Oocytes"), should not be set too generously (see chapter "Indications for and Against Fertility Preservation").

Cryopreservation of Ovarian Tissue Versus Cryopreservation of Oocytes

Cryopreservation of ovarian tissue as well as ovarian stimulation and cryopreservation of oocytes is possible for most women. In this case, the decision is often based on the technical and logistical capabilities of the centre. However, there are also factors and criteria, where one of the techniques should be preferred. Under certain conditions, only one of the techniques is definitely feasible (Table 4).

The easier use of frozen oocytes due to the laparoscopy required to transplant ovarian tissue is probably the reason why frozen oocytes (see chapter "Cryopreservation of Unfertilized and Fertilized Oocytes") are more often used to generate a pregnancy that frozen ovarian tissue (Table 3).

In the case of highly gonadotoxic treatments, ovarian stimulation/cryopreservation of oocytes can be combined with removal/cryopreservation of ovarian tissue. Although both procedures can be carried out in succession, we strongly recommend primary removal/cryopreservation of the tissue and only then subsequent ovarian stimulation, as tissue quality is very low just after stimulation. Since the oocyte quality is not negatively influenced by previous tissue removal, it has become common practice to first remove approximately 50% of an ovary and then (i.e. after 2–3 days) to start ovarian stimulation (see chapter "Ovarian Stimulation to Collect Oocytes").

Practical Approach

Prior to Removal

Before removal of ovarian tissue, the antral follicle count (AFC) should be determined by ultrasound measurement, ideally supplemented by measuring the serum concentration of anti-Müllerian hormone (AMH) to assess the ovarian reserve. Cryopreservation of tissue can thus be avoided if the ovarian reserve is limited. Preoperatively, it should also be checked whether and if so on which side a follicle is maturing or whether a corpus luteum or a pathological change can be found. The ovarian tissue on the side of the corpus luteum or pathological alteration should rather not be removed, as the risk of bleeding is greater and the quality of the ovarian tissue may also be lower.

It must also be ensured that there is no hepatitis B/C or HIV infection. Exclusion of *Treponema pallidum* infection is also required in Germany.

Removal

Due to the unclear effect of methylene blue on follicles and oocytes, saline solution should preferably be used for the intraoperative examination of tube patency. If there is unilateral tube closure, a tissue sample should be taken from this side. Tissue removal should not be performed on the side of the open tube so that the chance of spontaneous pregnancy is not reduced.

An entire ovary or about 50% of an ovary is removed. Tissue is usually removed on an outpatient basis by laparoscopy if the patient's state of health permits. Unilateral ovariectomy is only necessary in children in who partial ovariectomy is

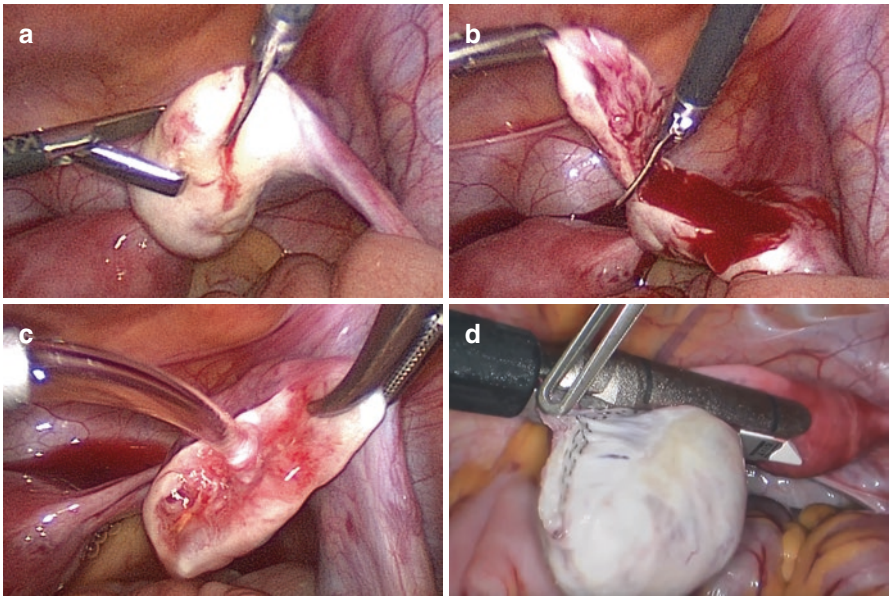


Fig. 1 (**a**-**c**): Laparoscopic hemiovariectomy: Single grasping of the surface of the ovary with grasping forceps (**a**), removal of half an ovary with sharp scissors with a straight, clean incision, if necessary after single repositioning of the grasping forceps (**b**), wound surface after punctual bipolar coagulation of the sources of bleeding with repeated irrigation of the wound surface (**c**), no wound closure (University Women's Hospital Bern); (**d**): Ovariectomy with an EndoGIA stapler (16, with permission of Catherine Poirot and Anne Fortin, Paris)

not possible due to the size of the ovary, or if highly gonadotoxic treatments are given with a high risk of complete loss of ovarian function. Accordingly, in the *Ferti*PROTEKT network, only 3% of women underwent unilateral ovariectomy in 2018 [15].

For an ovariectomy, the supplying ovarian vessels are ligated, and the ovary is then removed without electrical coagulation. Alternatively, a stapler (Fig. 1) [16] can be used.

To remove half an ovary, the outer pole is grasped with a pair of forceps. Approximately 50% of the tissue is removed antimesenterically with a smooth incision (to minimize bleeding), retrieved via a 12-mm trocar and immediately stored in a prepared transport medium. The latter is pre-cooled to 4–8 °C.

Care should be taken if there is bleeding from the ovarian medulla (the central areas of the wound surface), as coagulation in this area can lead to damage of the supplying vessels and thus necrosis of the rest of the ovary. Coagulation of the wound surface and sutures to close the wound area are not necessary. Bilateral removal of ovarian tissue should be avoided because of the risk of periovarian adhesions that can lead to a reduction in fertility.

References

1. Donnez J, Dolmans MM. Fertility preservation in women. N Engl J Med. 2017;377:1657–65.
2. Corkum KS, Rhee DS, Wafford QE, Demeestere I, Dasgupta R, Baertschiger R, et al. Fertility and hormone preservation and restoration for female children and adolescents receiving gonadotoxic cancer treatments: a systematic review. J Pediatr Surg. 2019;54:2200–9.
3. von Wolff M, Stute P, Flück C. Autologous transplantation of cryopreserved ovarian tissue to induce puberty-the endocrinologists' view. Eur J Pediatr. 2016;175:2007–10.
4. Deutsche Gesellschaft für Gynäkologie und Geburtshilfe (DGGG), Deutsche Gesellschaft für Reproduktionsmedizin (DGRM), Deutsche Gesellschaft für Urologie (DGU). Leitlinie: Fertilitätserhaltung bei onkologischen Therapien. Level S2k, AWMF Register Nr. 015/082, November 2017. http://www.awmf.org/leitlinien/detail/ll/015-082.html.
5. Dolmans MM, Masciangelo R. Risk of transplanting malignant cells in cryopreserved ovarian tissue. Minerva Ginecol. 2018;70:436–43.
6. Shapira M, Raanani H, Barshack I, Amariglio N, Derech-Haim S, Marciano MN, et al. First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination. Fertil Steril. 2018;109:48–53.
7. Gook D, Westerman D, McBean M, Hughes V, Stern K. Potential leukaemic contamination in cryopreserved ovarian tissue. Hum Reprod. 2018;33(Suppl 1):i38–9.
8. Poirot C, Fortin A, Lacorte JM, Akakpo JP, Genestie C, Vernant JP, CAROLéLISA Cooperative Group, et al. Impact of cancer chemotherapy before ovarian cortex cryopreservation on ovarian tissue transplantation. Hum Reprod. 2019;34:1083–94.
9. Meirow D, Ra'anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertil Steril. 2016;106:467–74.
10. Lawrenz B, Jauckus J, Kupka MS, Strowitzki T, von Wolff M. Fertility preservation in >1,000 patients: patient's characteristics, spectrum, efficacy and risks of applied preservation techniques. Arch Gynecol Obstet. 2011;283:651–6.
11. von Wolff M, Dittrich R, Liebenthron J, Nawroth F, Schüring AN, Bruckner T, et al. Fertility-preservation counselling and treatment for medical reasons: data from a multinational network of over 5000 women. Reprod Biomed Online. 2015;31:605–12.
12. Van der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krüssel J, et al. FertiPROTEKT network. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. Hum Reprod. 2016;31:2031–41.
13. Jadoul P, Guilmain A, Squifflet J, Luyckx M, Votino R, Wyns C, et al. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. Hum Reprod. 2017;32:1046–54.
14. Diaz-Garcia C, Domingo J, Garcia-Velasco JA, Herraiz S, Mirabet V, Iniesta I, et al. Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for adult women undergoing gonadotoxic treatments: a prospective cohort study. Fertil Steril. 2018;109:478–85.e2.
15. Germeyer A, Dittrich R, Liebenthron J, Nawroth F, Sänger N, Suerdieck M, von Wolff M. D.I.R. annual report 2018. J Reprod Med Endocrinol. 2019;16:41–4.
16. Fortin A, Azaïs H, Uzan C, Lefebvre G, Canlorbe G, Poirot C. Laparoscopic ovarian tissue harvesting and orthotopic ovarian cortex grafting for fertility preservation: less is more. Fertil Steril. 2019;111:408–10.

Transport, Cryopreservation and Storage of Ovarian Tissue

Jana Liebenthron

Introduction

According to current knowledge, cryopreservation of ovarian tissue is carried out by slow freezing with automatic seeding, whereby controlled production and maintenance of an equilibrium between the intracellular water and the unfrozen water takes place in the low concentration cryoprotective agent. During the slow, precisely defined cooling, dehydration of the cells occurs, thereby preventing intracellular ice crystal formation and thus irreversible cell damage. The time to equilibrium depends on various factors, such as the cell volume of the individual cells in the tissue, the ratio of surface area to volume and the permeability of the cell membrane, which determines a tissue-specific freezing rate. The aim is a reversible arrest of the cell metabolism at −196 °C, preservation of the structure of the germ cells embedded in the follicle and their genetic integrity, acceptable survival rates after cryopreservation and thawing and reproducible results.

Storage and Transport of the Removed Tissue Until Preparation and Cryopreservation

The ovarian tissue should be transferred into a sterile, certified transport medium immediately after removal (e.g. Custodiol®, Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) and transported at 4–8 °C to the processing site, where high-quality, standardized processing, cryopreservation and storage of ovarian tissue can be guaranteed by professional expertise and technology. This tissue facility should

J. Liebenthron (✉)
UniCareD, University Cryobank for Assisted Reproductive Medicine and Fertility Protection at UniKiD Duesseldorf, University Hospital Duesseldorf, Duesseldorf, Germany
e-mail: Jana.Liebenthron@unicared.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_22

ensure that the processing or preparation, including labelling, cryopreservation and storage as well as testing are carried out in accordance with current knowledge and technology (Section "Tissue Bank Requirements for the Preparation, Cryopreservation and Storage of Ovarian Tissue").

If preparation/storage and cryopreservation of ovarian tissue cannot be carried out directly at the place of removal, the *Ferti*PROTEKT network offers the possibility of cooperating with specialized, external and centralized cryobanks.

The transport there is carried out directly post-operatively in special transport containers, which ensure that the cold chain is uninterrupted (Fig. 1). Studies have shown that hypothermia of the resected ovarian tissue can last up to 24 h (i.e. overnight if necessary) in different transport periods without lasting damage to the follicle reserve or the success rate after transplantation being adversely affected [1–3]. Temperature loggers continuously record the temperature in the transport containers. The advantage of this system is its robustness and easy handling when sending the stable transport containers. The disadvantage is the need for optimal logistics and organization in the dispatch procedures from the tissue removal centres/clinics according to the specifications of the centralized cryobanks. A recent study of the methods [2] illustrates this and shows that suboptimal transport conditions for the samples can occur—caused by incorrect handling and storage of the cooling elements, which allow a stable temperature gradient of 4–8 °C for 24 h in the boxes. The high pregnancy rates after transplantation of tissue transported in this way as well as vitality tests on the transported tissue demonstrate that the transport system functions well. There are no detectable restrictions compared to non-transported tissue [2].

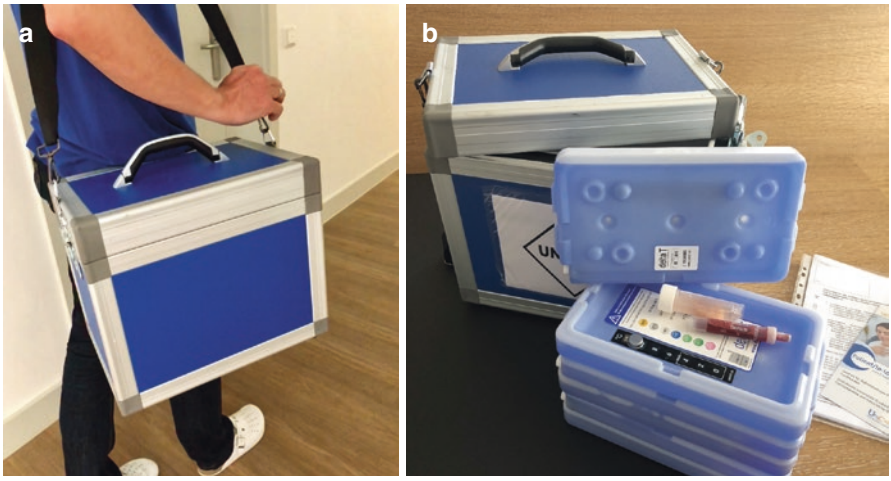


Fig. 1 (**a**) Transportation box for overnight transportation. (**b**) Contents of the box: three pairs of cooling elements (precooled at 2–8 °C), temperature logger for monitoring transport conditions, flask with transportation medium and ovarian tissue, blood for analysing anti-Müllerian hormone concentration (AMH) in serum and instruction sheets

Considering the transport logistics of centralized cryobanks internationally, Denmark, for example uses a transport system in which the removed tissue is transported in a sealed container at about 0 °C on ice. The tissue is shipped by land and air and reaches the cryobank after a maximum of 4–5 h [4]. The advantage of this system is that technical errors with a resulting too high transport temperature are unlikely due to the stable temperature of ice. The pregnancy rates after the transplantation of transported tissue in Denmark [5] also allow the conclusion that this transport system also does not lead to relevant tissue damage [1].

Both the multiplication rates and the pregnancy and birth rates of both systems are comparable to other internationally published success rates.

Tissue Preparation

Preparation must be carried out in a sterile Class II laminar airflow cabinet. The laminar air flow should stand in a clean room of cleanliness level 'ISO Class 7/GMP Grade B "in operation", Grade C "at rest"'. Processing should ideally take place in a separate laboratory, accessible via an airlock, in a contamination-free environment in which only ovarian tissue is aseptically prepared and cooled (e.g. using an integrated cooling plate, UKH602, FRYKA Kältetechnik GmbH, Esslingen, Germany).

During preparation, the ovarian medulla should be carefully separated from the ovarian cortex using precision scalpels and anatomical forceps (Fig. 2), leaving a thin residual stroma so that an optimal starting point for neovascularization of the grafts is available later [6]. Depending on the size and quality, rectangular cortex pieces measuring approximately $4 \times 8 \times 1$ mm^3 are cut from the prepared cortex, cooled in a suitable medium for cryopreservation, equilibrated and then transferred into individual cryotubes filled with the same cryopreservation medium [2, 7–9].

Cryopreservation and Storage

The samples are cooled using a computer-controlled slow freezing procedure (e.g. using an IceCube 14S-A, SY-LAB, Neupurkersdorf, Austria) according to a modified program by Gosden et al. [10] so that they can then be stored permanently in a nitrogen gas phase (at −196 °C).

The selection of a suitable cryoprotectant is an important prerequisite and has a significant influence on the successful course of a cryopreservation technique. The best survival rates of embedded germ cells as well as intact tissue morphologies and cell structures can be demonstrated after cryopreservation and thawing with DMSO in combination with an appropriate carrier medium and a protein additive [2, 7–9].

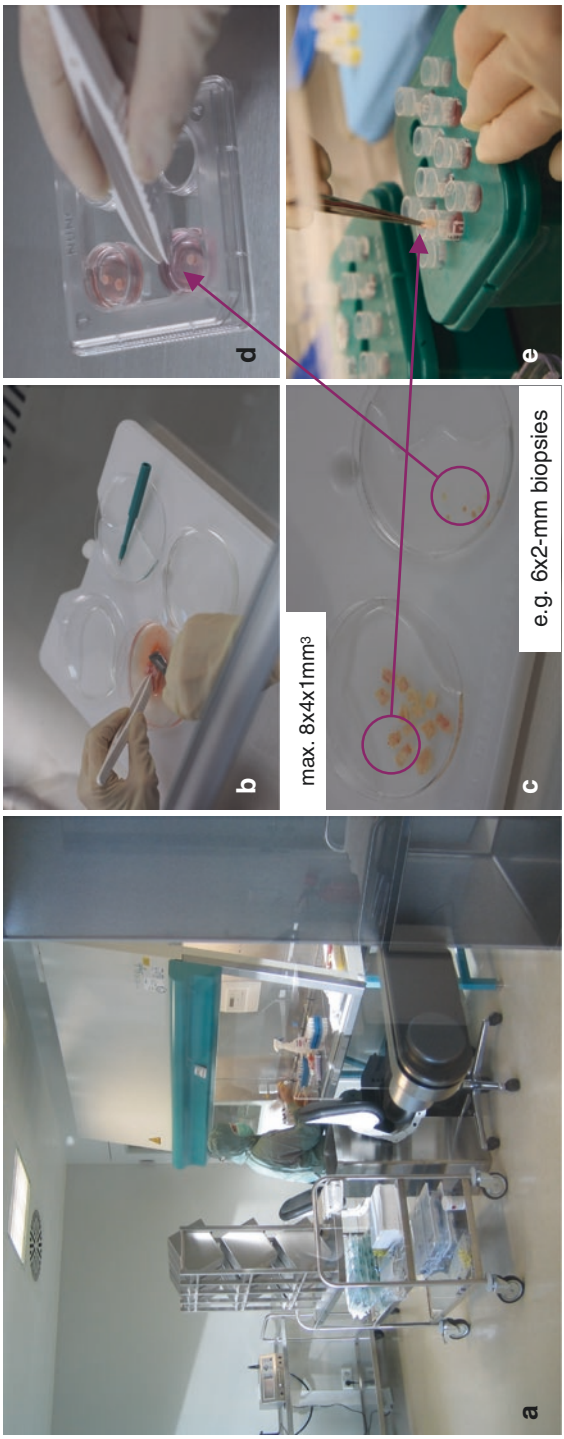


Fig. 2 (**a**) Clean room laboratory for preparation of ovarian tissue. (**b**) Preparation under cooled conditions (2–4 °C) to avoid interruption of the cooling chain after transportation to reduce cell metabolism. (**c**) Preparation and (**d**) digestion of standardized biopsies for quality control. (**e**) Transferring tissue into cryo tubes for cryopreservation by slow freezing

Quality Control

Determination of the Ovarian Cortex Potential Before and After Cryopreservation

The higher the ovarian cortex reserve at the time of removal and before the start of oncological therapy, the greater the success of this fertility-preserving method, that is the chance of a later pregnancy and birth of a healthy child from the transplants. The ovarian reserve can be estimated relatively precisely by ultrasound determination of the AFC (antral follicle count), the size of the ovary, the AMH (anti-Müllerian hormone) concentration, the age and the additional determination of the primordial and primary follicle density in the transplants to be prepared using suitable detection methods.

For this, standardized biopsies are taken from different tissue margins during tissue preparation (e.g. $6 \times 2 \times 1$ mm^3 biopsies [11]). These are then divided into two portions of equal size ($3 \times 2 \times 1$ mm^3 each), are both freshly examined and frozen, to be later thawed and examined using a vitality assay, e.g. a calcein–acetoxymethyl ester AM stain, [11] (Fig. 3) [2, 8, 9].

These analysis results from fresh- and thaw tests can be summarized, averaged and correlated with the age of the patient at the time of tissue removal, the AMH value and the AFC, and the number of tissue strips to be transplanted can be determined. As a rule, the amount of ovarian tissue to be transplanted corresponds to approximately 15–20% of a whole ovary (one ovary = 100%). Assuming that 50% of the ovary has been removed from the patient from which 10 tissue strips measuring approximately $4 \times 8 \times 1$ mm^3 have been prepared and cryopreserved, three strips are thawed and transplanted in the first transplantation. If the follicle density is low and the patient is older at the time of removal (ca. >30 years), the number of tissue strips to be transplanted should be increased to 4–5, corresponding to 20–25% of the ovary (1 ovary = 100%) [11].

These tests also ensure that the tissue removal, its transport, preparation and cryopreservation have not led to any relevant damage (loss of follicles and oocytes) [2, 8].

In addition, in vitro tests can also be performed which reflect the overall tissue functionality, since only a completely intact ovarian cortex can supply the follicles sufficiently and support their growth. Both the measurement of the in vitro glucose consumption of standardized cortex biopsies before and after cryopreservation (glucose-uptake assay) and the determination of estradiol and progesterone in the medium supernatant of cultivated cortex biopsies represent such tests [12].

Validation of an Ovarian Tissue Processing Facility

This validation ensures the quality of the preparation, cryopreservation and thawing at each individual centre with the aim of being able to offer an optimal and consistent standard.

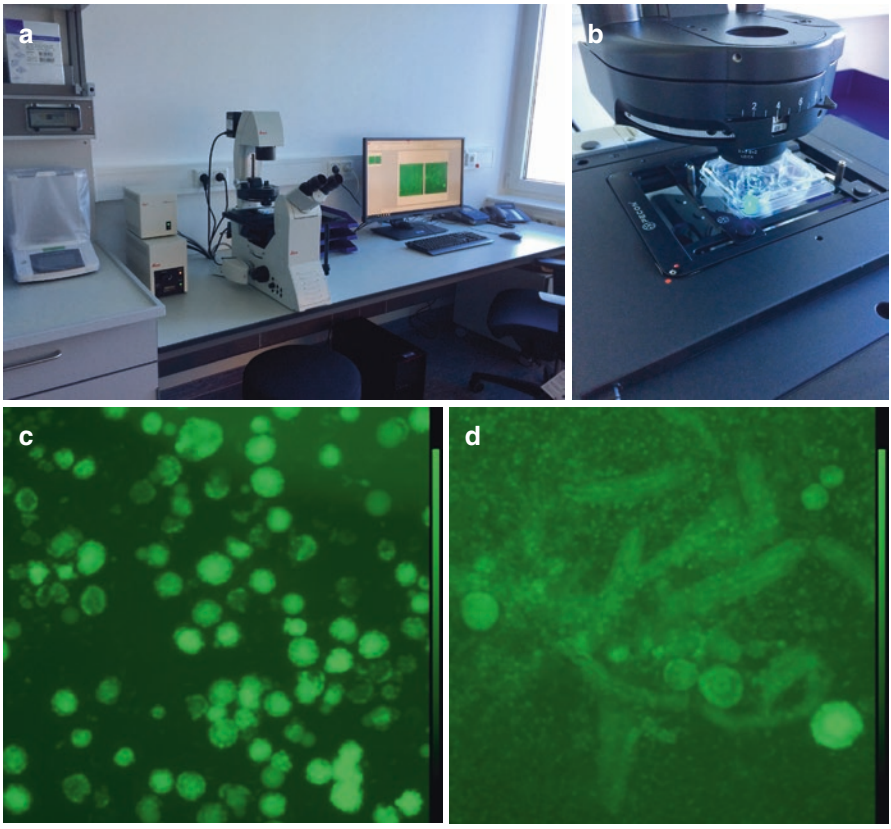


Fig. 3 (**a**) Assessment of ovarian cortex potential—before and after cryopreservation of standardized biopsies using an inverse fluorescence microscope. (**b**) 3 × 2 mm biopsies in each well after digestion with collagenase and staining with calcein AM. (**c**) Viable/fluorescent primordial and primary follicles from a prepubertal patient. (**d**) Blood vessels and low numbers of follicles from ovarian cortex with a corpus luteum

The *Ferti*PROTEKT network therefore recommends that cryopreserving centres have their freezing technique checked by xenotransplantation of ovarian tissue into immunodeficient SCID (severe combined immunodeficiency) mice before offering transplantation in clinical routine. The aim is to show that the follicles have good development potential after cryopreservation and thawing.

Tissue Bank Requirements for the Preparation, Cryopreservation and Storage of Ovarian Tissue

- The tissue facility must ensure that tissue processing, including labelling, cryopreservation, storage and testing, is carried out in accordance with current scientific and technical knowledge.

- The preparation must be performed in a sterile Class II laminar air flow cabinet [7, 8]. The laminar air flow should be located in a clean room of cleanliness level "ISO Class 7/GMP Grade B 'in operation', Grade C 'at rest'" [13]. Ideally, processing should take place in a separate laboratory, accessible via an airlock, in a contamination-free environment in which only ovarian tissue is aseptically prepared and cooled.
- In accordance with GMP criteria (Good Manufacturing Practice), it must be ensured that the patient's infection status is negative before collection (Exclusion of hepatitis B and C and HIV infection. Exclusion of *Treponema pallidum* infection is also required in Germany).
- Additional measurement of the current AMH concentration in serum prior to tissue removal, ultrasound measurement of the ovaries and the AFC, the age of the woman and the density of the primordial and primary follicles serve to estimate the ovarian reserve and therefore how much ovarian tissue should later be transplanted (see section "Determination of the Ovarian Cortex Potential Before and After Cryopreservation") [7, 8].
- The establishment of a method for determining the density of primordial and primary follicles to verify that collection, transport, preparation and cryopreservation have not led to any relevant damage to the follicles and oocytes [9].
- Submitted ovarian tissue material should not be released for use until its quality can be assessed as satisfactory and can be demonstrated accordingly in writing. When tissue is removed, a small sample must also be taken to histopathologically exclude malignant cells/metastases [7, 8].
- A verifiable quality management system should ensure that these requirements are met.

References

1. Schmidt KL, Ernst E, Byskov AG, Nyboe AA, Yding AC. Survival of primordial follicles following prolonged transportation of ovarian tissue prior to cryopreservation. Hum Reprod. 2003;18(12):2654–9. https://doi.org/10.1093/humrep/deg500.
2. Liebenthron J, Montag M, Reinsberg J, Köster M, Isachenko V, van der Ven K, et al. Overnight ovarian tissue transportation for centralized cryobanking: a feasible option. Reprod Biomed Online. 2019;38(5):740–9. https://doi.org/10.1016/j.rbmo.2019.01.006. Epub 2019 Jan 19
3. von Wolff M, Andersen CY, Woodruff TK, Nawroth F. *Ferti*PROTEKT, Oncofertility consortium and the Danish fertility-preservation networks - what can we learn from their experiences? Clin Med Insights Reprod Health. 2019;13:1179558119845865. https://doi.org/10.1177/1179558119845865. eCollection 2019
4. Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, et al. Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique. Reprod Biomed Online. 2011;22(2):162–71. https://doi.org/10.1016/j.rbmo.2010.10.015. Epub 2010 Nov 16
5. Gellert SE, Pors SE, Kristensen SG, Bay-Bjørn AM, Ernst E, Yding AC. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. J Assist Reprod Genet. 2018;35(4):561–70. https://doi.org/10.1007/s10815-018-1144-2. Epub 2018 Mar 1
6. Donnez J, Dolmans MM. Ovarian tissue freezing: current status. Curr Opin Obstet Gynecol. 2015;27(3):222–30. https://doi.org/10.1097/GCO.0000000000000171. Review

7. Bastings L, Liebenthron J, Westphal JR, Beerendonk CC, van der Ven H, Meinecke B, et al. Efficacy of ovarian tissue cryopreservation in a major European center. J Assist Reprod Genet. 2014;31(8):1003–12. https://doi.org/10.1007/s10815-014-0239-7. Epub 2014 Jun 14
8. Beckmann MW, Lotz L, Toth B, Baston-Büst DM, Fehm T, Frambach T, et al. Concept paper on the technique of cryopreservation, removal and transplantation of ovarian tissue for fertility preservation. Geburtshilfe Frauenheilkd. 2019;79(1):53–62. https://doi.org/10.1055/a-0664-8619. Epub 2018 Oct 9
9. Deutsche Gesellschaft für Gynäkologie und Geburtshilfe (DGGG), Deutsche Gesellschaft für Reproduktionsmedizin (DGRM), Deutsche Gesellschaft für Urologie (DGU). Leitlinie: Fertilitätserhaltung bei onkologischen Therapien. Level S2k, AWMF Register Nr. 015/082, November 2017. http://www.awmf.org/leitlinien/detail/ll/015-082.html.
10. Gosden RG, Baird DT, Wade JC, Webb R. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at −196°C. Hum Reprod. 1994;9:597–603. https://doi.org/10.1093/oxfordjournals.humrep.a138556.
11. Liebenthron J, Reinsberg J, van der Ven H, Saenger N, Kruessel JS, von Wolff M. Serum anti-Mullerian hormone concentration and follicle density throughout reproductive life and in different diseases - implications in fertility preservation. Hum Reprod. 2019;34(12):2513–22. https://doi.org/10.1093/humrep/dez215.
12. Isachenko E, Isachenko V, Nawroth F, Rahimi G, Weiss JM. Effect of long-term exposure at suprazero temperatures on activity and viability of human ovarian cortex. Fertil Steril. 2009;91(4 Suppl):1556–9. https://doi.org/10.1016/j.fertnstert.2008.09.068. Epub 2008 Nov 20
13. Mortimer D, Cohen J, Mortimer ST, Fawzy M, McCulloh DH, Morbeck DE, et al. Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting. Reprod Biomed Online. 2018;36(6):658–74. https://doi.org/10.1016/j.rbmo.2018.02.005. Epub 2018 Mar 2

Transplantation of Ovarian Tissue

Michael von Wolff

Introduction

Cryopreserved ovarian tissue can be transplanted and used in vivo and in vitro to generate oocytes. It is also possible to use the tissue's hormone production to induce puberty or as a temporary substitute for hormone replacement therapy.

Generation of oocytes in vitro by maturation of the primordial follicles is still experimental and is described in chapter "Further Fertility Preservation Techniques". Using the tissue to induce puberty or as a temporary replacement for hormone replacement therapy is at first glance an attractive, apparently physiological option. However, this technique is controversial because of its many disadvantages (see section "Transplantation to Avoid Hormone Replacement Therapy").

Intra-abdominal transplantation of ovarian tissue to generate spontaneous or IVF-assisted pregnancies is recognized in many countries, as it offers many advantages. Transplantation into the abdomen is preferred because the development of the follicles intra-abdominally is most favourable due to the pressure and temperature conditions there. Transplantation is ideally carried out orthotopically, that is into the pelvis in an area that allows spontaneous pregnancy (Table 1).

M. von Wolff (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_23

Table 1 Possible advantages and disadvantages of ovarian tissue transplantation

Advantages of transplantation	Disadvantages of transplantation
Spontaneous pregnancy possible	Pregnancy chances after transplantation cannot yet be precisely quantified due to limited data
Repeated transplantations possible	Transplantation of ovarian tissue not yet clinically established, optimal location of the graft, surgical technique and required amount of ovarian tissue are still unclear

Effectiveness and Risks

Effectiveness

The data available for assessing the effectiveness of ovarian tissue transplantation is still limited. However, since the data from case series published to date show similar success rates, the birth rate per transplant can already be estimated. According to the larger studies published since 2016 (Table 2), one in four women will have a child after a transplant.

Most pregnancies following ovarian tissue transplantation occur spontaneously, which is why the tissue should be located in the ovarian fossa or ovary. According to the studies presented in Table 2, the spontaneous pregnancy rate is 66.7%. Gellert et al. [5] determined a spontaneous pregnancy rate of 46% in a worldwide survey of transplant centres. When interpreting the data, it should be noted that it cannot be completely excluded that the pregnancies may also originate from non-transplanted ovarian tissue.

The preference of spontaneous pregnancy instead of IVF therapy also makes sense for the following reasons:

Data from Natural cycle (NC) IVF therapies [7] show that the pregnancy rate per transfer in NC-IVFs in women <35 years is approximately 30%. However, since only every second cycle leads to a transfer, the pregnancy rate per cycle is only about 15% and is therefore only half as high as in spontaneous cycles. Therefore, monofollicular IVF without indication for IVF therapy is less effective than an attempt of spontaneous pregnancy. In addition, the effectiveness of IVF after ovarian tissue transplantation is lower in most hypergonadotropic women than in normal normogonadotropic women [2, 8]. It is probably more sensible to initially aim for spontaneous pregnancy if the patient has regular cycles, open fallopian tubes and normozoospermia.

Van der Ven et al. 2016 [1] analysed success rates depending on the woman's age at cryopreservation. These data show a strong age dependency of the success rate with a significant decrease in women ≥35 years. In women <35 years it was 37.8% and in women ≥35 years only 15.4%.

Transplants are performed for many different diseases, with various techniques and under different conditions, as demonstrated by some of the transplants in the *Ferti*PROTEKT network [22] (Table 3).

A clear correlation of the success rate with the amount of transplanted tissue and the follicle density has not yet been proven [9].

Table 2 Pregnancy and birth rates after transplantation of ovarian tissue

	Transplanted patients, *n*	Patients with ovarian tissue activity after transplantation, *n* (%)	Total pregnancies, *n*	Patients with at least one pregnancy, *n* (%)	Patients with at least one spontaneous pregnancy, *n* (%)	Patients with at least one live birth, *n* (%)
Van der Veen et al. 2016 (Germany, (*Ferti*PROTEKT) [1]	49	33 (67.3%)	21	16 (32.7%)	13	15 (30.3%)
Meirow et al. 2016 (Israel) [2]	20	19 (95%)	16	10 (50.0%)	3	6 (30%)
Jadoul et al. 2017 (Belgium) [3]	21	No data	No data	7 (33.3%)	No data	7 (33.3%)
Diaz-Garcia et al. 2018 (Spain, IVI) [4]	44	20 (45.4%)	15	12 (27.3%)	7	8 (18.2%)
Gellert et al. 2018 (Denmark) [5]	89	No data	33	23 (25.4%)	No data	16 (18%)
Fortin et al. 2019 (France) [6]	34	30 (88.2%)	15	10 (29.4%)	9	10 (29.4%)
Total	**257**	**102/147 (69.4%)**		**72/257 (28.0%)**	**32/48 (66.7%)**	**62/257 (24.1%)**

Table 3 Transplants in the *Ferti*PROTEKT network after overnight transport of the tissue prior to cryopreservation in 14 patients with premature ovarian insufficiency (POI) that led to at least one birth [22]

	Diagnosis	Age at cryopreservation (years)	Age at transplantation (years)	AMH before cryopreservation (ng/mL)	Chemo-therapy	Transplantation site	Pregnancies, (*n*)	Births (*n*)
1	Breast cancer	33	38	0.54	Yes	Peritoneal pouch	1	Consecutive
2	SLE	26	32	1.31	Yes	Peritoneal pouch	1	1 (twins)
3	Hodgkin's lymphoma	27	32	No data	Yes	Peritoneal pouch	1	1
4	Hodgkin's lymphoma	35	37	0.96	Yes	Peritoneal pouch	1	1
5	Hodgkin's lymphoma	21	27	2.25	Yes	Peritoneal pouch	1	1
6	Breast cancer	34	38	0.54	Yes	Peritoneal pouch	2	1 (1× miscarriage)
7	Hodgkin's lymphoma	33	37	0.83	Yes	Peritoneal pouch	2	2
8	Cystadenofibroma	20	27	No data	No	Peritoneal pouch	2 (1× IVF)	2 (1× IVF, 1× spontaneous and twins)
9	Breast cancer	36	37	3.61	Yes	Peritoneal pouch	1	1
10	Hodgkin's lymphoma	30	32	2.43	Yes	Ovary, peritoneal pouch	1	1
11	Borderline ovarian tumour	21	28	No data	Yes	Peritoneal pouch	1 (IVF)	1 (IVF)
12	Ewing's sarcoma	26	29	8.02	Yes	Peritoneal pouch	1	1
13	Breast cancer	36	39	1.06	Yes	Peritoneal pouch	1	1
14	Breast cancer	33	36	5.53	Yes	Peritoneal pouch	1	1

A birth rate of 50% per transplanted woman appears realistically achievable with optimized transplantation techniques and after repeated transplantations of tissue cryopreserved at the age of <35 years; however, this must still be confirmed in future studies.

Risks

Transplantation of ovarian tissue requires a laparoscopy, possibly also a laparotomy. The transplantation is usually performed laparoscopically. Transplantation into the pelvic peritoneum (Fig. 1) is always possible by laparoscopy. Transplantation onto the ovary can also be carried out laparoscopically (Fig. 4) or robot-assisted [10], but high surgical expertise is required. The risk of surgery when transplanting ovarian tissue is no higher than with any other laparoscopy, since women generally have a good state of health at the time of surgery. If the transplantation is performed by laparotomy, the surgical risks are slightly higher, convalescence is longer, and the cosmetic results are not as good as after laparoscopy.

The risks of malignant cell transmission were described in chapter "Removal of Ovarian Tissue".

Techniques to Improve the Effectiveness of Transplantation

Ovarian tissue can be transported and cryopreserved (see chapter "Transport, Cryopreservation and Storage of Ovarian Tissue") apparently without a relevant reduction in primordial follicle density. The tissue only loses a large part of the primordial follicles after the transplantation due to postoperative ischemia, caused by the initial lack of vascularization of the tissue. Therefore, various measures have been proposed to minimize this reduction (Table 4). However, these are still experimental and further studies must be awaited.

Transplantation to Avoid Hormone Replacement Therapy

Since the transplanted ovarian tissue is active for several years [17] and cyclically produces estradiol through the associated folliculogenesis, it is possible to use this tissue as a kind of "tissue hormone replacement therapy" (TRT) [18] instead of conventional "hormone replacement therapy" (HRT). However, endocrinologists [18] are critical of this procedure because:

- Surgery is always necessary for transplantation.
- The physiological production of progesterone by the tissue in women without a uterus is not necessary and is even counterproductive from a health point of view.

Table 4 Experimental techniques in animals to improve transplant effectiveness

Transplant treated with:	Rationale	Effect
Vascular endothelial growth factor (VEGF) [11]	Improvement of angiogenesis	Primordial follicle survival rate ↑ Vascular density in the graft ↑
Gonadotrophin (HMG) [12]	Optimization of folliculogenesis	Number of surviving follicles ↑ Vascularization ↑ VEGF-expression ↑
Anti-Müllerian hormone (AMH) [13]	Reduction in follicle recruitment	Percentage of primordial follicles ↑
Apoptosis inhibitors e.g. sphingosine 1-phosphate (S1P) [14]	Reduction of apoptosis	Number of apoptotic follicles ↓ Vascular density ↑
Co-transplantation with stem cells [15]	Improvement of angiogenesis	Number of surviving follicles ↑ Vascularization ↑
Patient treated with antioxidants, e.g. melatonin [16]	Reduction of oxidative activity	Follicle survival rate ↑

- The amount of hormone cannot be controlled.
- Hormone secretion can only be stopped by another operation.
- The procedure is considerably more expensive than drug-based hormone replacement therapy.
- Furthermore, the risk of transmitting malignant cells should be considered.

The same applies to the transplantation of ovarian tissue to induce puberty [19]. Therefore, these procedures should be described as experimental and should only be used under study conditions.

Practical Approach

Introduction

There are different surgical approaches and techniques. With increasing invasiveness, these include:

- laparoscopy (with or without the use of a surgical robot)
- mini laparotomy and
- laparotomy

Ideally, the least invasive approach should be chosen without reducing the effectiveness of the transplant.

There are also various transplant locations. With increasing similarity to natural localization, these include:

- subperitoneal heterotopic transplantation into the abdominal wall
- orthotopic transplantation into the broad ligament of the uterus
- orthotopic transplantation into the lateral pelvic peritoneum
- orthotopic transplantation into the ovary and
- orthotopic transplantation on the decorticated ovarian surface

However, this does not imply that the best transplant location in accordance with natural conditions is also the best. For example, a transplant into the well-perfused pelvic peritoneum may be better than a transplant into or onto an atrophic ovary.

The preferred techniques of transplantation and localization of the graft are still unclear. The surgical procedure depends strongly on the transplant centre's surgical expertise and technical equipment.

Therefore, only a few possible surgical techniques are presented here. Further techniques and instructive videos can be found as "peer reviewed published" videos on YouTube [6, 10].

Prior to Transplantation

The tissue is thawed by specially trained biologists shortly before transplantation. It is still unclear how much ovarian tissue should be transplanted. In the transplants that led to pregnancy in the *Ferti*PROTEKT network [1], after cryopreservation of approximately 50% of an ovary, usually 1/3 to 1/2 of the preserved tissue (approximately 15–20% of an ovary; one ovary = 100%) was transplanted. Other centres transplant larger amounts of tissue, especially after cryopreservation of a whole ovary.

During the transplantation, the patency of the tubes should be checked, and a hysteroscopy should be considered if necessary.

Transplantation into the Pelvic Peritoneum (Figs. 1 and 2)

The parietal peritoneum is incised lateral to the ovary over a length of approximately 0.5–1 cm. A subperitoneal pouch is bluntly prepared and the pieces of tissue inserted so that the cortex surfaces face the uterus. The pieces of tissue should lie next to each other and not on top of one other. The peritoneum is usually closed with a single suture (e.g. with PDS 5–0). If the pouch is deep enough, closure may not be necessary.

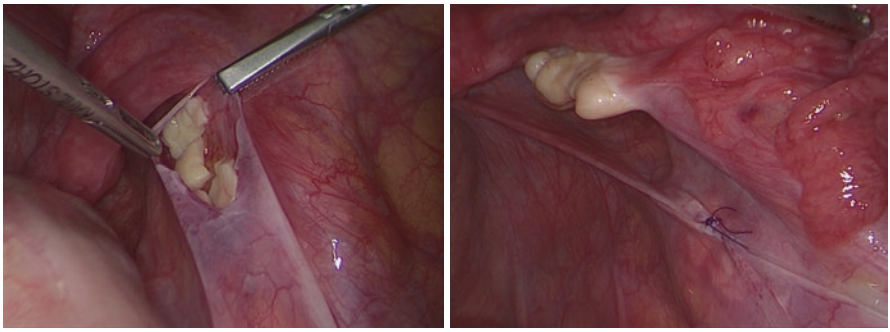


Fig. 1 Subperitoneal transplantation of ovarian tissue into the peritoneal wall (University Women's hospital Bern, Switzerland)

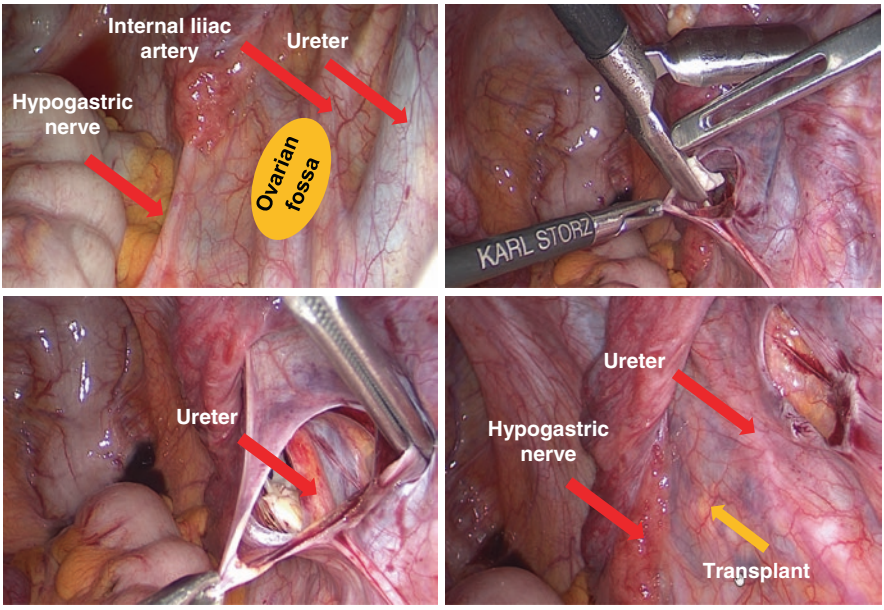


Fig. 2 Detailed illustration of subperitoneal transplantation of ovarian tissue into the peritoneal wall (University Women's hospital Bern, Switzerland)

Transplantation into the Ovary (Fig. 3)

The tissue is ideally transplanted into the larger ovary and thus usually into the organ from which the ovarian tissue was not removed before gonadotoxic treatment. The ovary is incised, resulting in a subcortical pouch. If this is not possible, it is incised centrally. If possible, the pieces of tissue are aligned in the pouch so that the cortex surfaces face the outer surface of the ovary. The ovary is usually closed with single sutures.

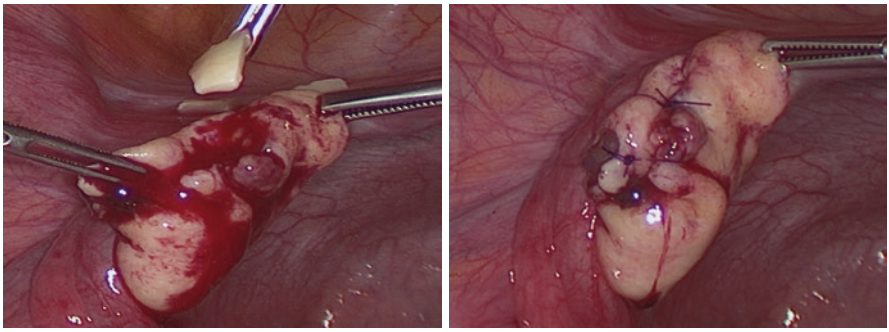


Fig. 3 Transplantation of ovarian tissue into the ovary (University Women's hospital Bern, Switzerland)

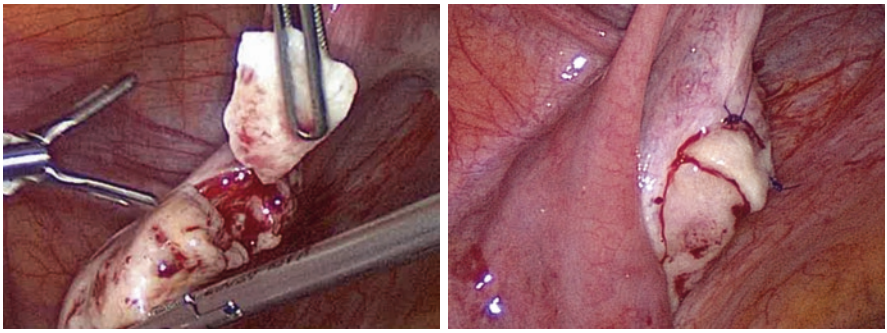


Fig. 4 Transplantation of ovarian tissue onto the ovary (University Women's hospital Bern, Switzerland)

Transplantation onto the Ovary (Fig. 4)

Transplantation onto the surface of the ovary requires the tissue pieces to be of sufficient size that they can be fixed. It has been described in the literature that the surface of the ovary is first decorticated before the tissue pieces are fixed [20]. However, this is hardly possible laparoscopically and if so, only with a smooth ovary surface. There is also a risk of losing a relevant amount of cortex, which is either still active. Therefore, the surface of the ovary can be incised with scissors, which opens a wound gap that is widened bluntly. The pieces of tissue can be fixed to these with single sutures (e.g. with PDS 5–0). Fixation with fibrin glue and covering with Interceed® mesh has been reported [21].

Follow-Up

The first signs of ovarian tissue activity appear after approximately 2–3 months but possibly also only after 6 months. Figure 5 shows an ectopic ovary after a subperitoneal transplant and the ovary after a transplant onto the ovary.

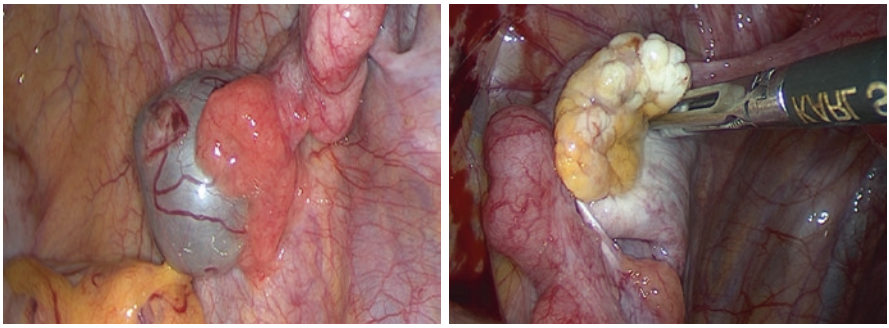


Fig. 5 Ovarian tissue after transplantation (left: follicle after subperitoneal transplantation into the peritoneal wall; right: after transplantation onto the ovary) (University Women's Hospital, Bern, Schweiz)

If there is tubal patency and no other relevant sterility factor, spontaneous pregnancy can be attempted. Cycle monitoring is often carried out, ovulation is induced with HCG and the timing of sexual intercourse is optimized. The many spontaneous pregnancies achieved confirm that spontaneous conception should be attempted for at least 6 months. If pregnancy does not occur or if there is another relevant infertility factor, IVF can be carried out, possibly combined with ICSI. Gonadotropin stimulation to generate several follicles can be tried, but this often does not lead to a multifollicular reaction due to the usually low ovarian reserve. Alternatively, modified "Natural Cycle IVF" can be performed [7].

Further details on follow-up after transplantation are described in chapter "Infertility Treatment After Fertility Preservation Therapies".

References

1. Van der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krüssel J, et al. *Ferti*PROTEKT network. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. Hum Reprod. 2016;31:2031–41.
2. Meirow D, Ra'anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertil Steril. 2016;106:467–74.
3. Jadoul P, Guilmain A, Squifflet J, Luyckx M, Votino R, Wyns C, et al. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. Hum Reprod. 2017;32:1046–54.
4. Diaz-Garcia C, Domingo J, Garcia-Velasco JA, Herraiz S, Mirabet V, Iniesta I, et al. Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for adult women undergoing gonadotoxic treatments: a prospective cohort study. Fertil Steril. 2018;109:478–85.e2.
5. Gellert SE, Pors SE, Kristensen SG, Bay-Bjørn AM, Ernst E, Yding AC. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. J Assist Reprod Genet. 2018;35:561–70.

6. Fortin A, Azaïs H, Uzan C, Lefebvre G, Canlorbe G, Poirot C. Laparoscopic ovarian tissue harvesting and orthotopic ovarian cortex grafting for fertility preservation: less is more. Fertil Steril. 2019;111:408–10.
7. von Wolff M. The role of Natural Cycle IVF in assisted reproduction. Best Pract Res Clin Endocrinol Metab. 2019;33:35–45.
8. Gook D, Hale L, Polyokov A, Stern K. Outcomes from heterotopic and orthotopic grafting of human cryopreserved ovarian tissue. Hum Reprod. 2019;34(Suppl 1):i60–1.
9. Jensen AK, Kristensen SG, Macklon KT, Jeppesen JV, Fedder J, Ernst E, et al. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. Hum Reprod. 2015;30:2838–45.
10. Oktay K, Taylan E, Kawahara T, Cillo GM. Robot-assisted orthotopic and heterotopic ovarian tissue transplantation techniques: surgical advances since our first success in 2000. Fertil Steril. 2019;111:604–6.
11. Shikanov A, Zhang Z, Xu M, Smith RM, Rajan A, Woodruff TK, et al. Fibrin encapsulation and vascular endothelial growth factor delivery promotes ovarian graft survival in mice. Tissue Eng Part A. 2011;17:3095–104.
12. Wang Y, Chang Q, Sun J, Dang L, Ma W, Hei C, Shen X, et al. Effects of HMG on revascularization and follicular survival in heterotopic autotransplants of mouse ovarian tissue. Reprod Biomed Online. 2012;24:646–53.
13. Man L, Park L, Bodine R, Ginsberg M, Zaninovic N, Man OA, et al. Engineered endothelium provides angiogenic and paracrine stimulus to grafted human ovarian tissue. Sci Rep. 2017;7:8203.
14. Soleimani R, Heytens E, Oktay K. Enhancement of neoangiogenesis and follicle survival by sphingosine-1-phosphate in human ovarian tissue xenotransplants. PLoS One. 2011;6:e19475.
15. Zhang Y, Xia X, Yan J, Yan L, Lu C, Zhu X, et al. Mesenchymal stem cell-derived angiogenin promotes primodial follicle survival and angiogenesis in transplanted human ovarian tissue. Reprod Biol Endocrinol. 2017;15:18.
16. Sapmaz E, Ayar A, Celik H, Sapmaz T, Kilic N, Yasar MA. Effects of melatonin and oxytetracycline in autologous intraperitoneal ovary transplantation in rats. Neuro Endocrinol Lett. 2003;24:350–4.
17. Donnez J, Squifflet J, Van Eyck AS, Demylle D, Jadoul P, Van Langendonckt A, et al. Restoration of ovarian function in orthotopically transplanted cryopreserved ovarian tissue: a pilot experience. Reprod Biomed Online. 2008;16:694–704.
18. von Wolff M, Stute P. Cryopreservation and transplantation of ovarian tissue exclusively to postpone menopause: technically possible but endocrinologically doubtful. Reprod Biomed Online. 2015;31:718–21.
19. von Wolff M, Stute P, Flück C. Autologous transplantation of cryopreserved ovarian tissue to induce puberty - the endocrinologists' view. Eur J Pediatr. 2016;175:2007–10.
20. Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. Fertil Steril. 2013;99:1514–22.
21. Donnez J, Dolmans MM. Transplantation of ovarian tissue. Best Pract Res Clin Obstet Gynaecol. 2014;28:1188–97.
22. Liebenthron J, Montag M, Reinsberg J, Köster M, Isachenko V, van der Ven K, van der Ven H, Krüssel JS, von Wolff M. Overnight ovarian tissue transportation for centralized cryobanking: a feasible option. Reprod Biomed Online. 2019;38:740–9.

GnRH Agonists

Frank Nawroth

Introduction

The use of gonadotropin-releasing hormone agonists (GnRHa) in the context of fertility preservation is based on the consideration that the resulting pituitary down-regulation and ovarian dysfunction could reduce the sensitivity of the ovarian tissue to cytotoxic effects.

However, primordial follicle activation is gonadotropin independent, which does not plausibly explain an influence on this pathway [1]. Since many chemotherapeutic drugs also influence non- or low-metabolic as well as actively dividing cells in the same way, this mechanism of action must be critically questioned.

There is currently no specific molecular explanation for the effective action of GnRHa on fertility preservation [2].

GnRHa can be used i.m. (once daily, monthly or as a 3-month depot), s.c. (once daily) or intranasally (two to three times daily). They bind to the specific pituitary receptor and lead to an initial "flair-up effect" over about 5–7 days, where short-term significantly increased gonadotropin secretion occurs. After the pituitary FSH/LH store empties, there is a decrease in the serum gonadotropin concentration, leading to the desired subsequent ovarian dormancy.

GnRH agonists (GnRHa) and GnRH antagonists (GnRHant) can be differentiated depending on their effect on the GnRH receptor of the pituitary gland. The latter leads to a blockade of the GnRH receptor and therefore of pituitary FSH and LH release within only about 8 h.

An initial combination of GnRHa with GnRHant did not completely prevent the flair-up effect but significantly reduced it in most patients [3].

F. Nawroth (✉)
Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology, amedes MVZ Hamburg, Hamburg, Germany
e-mail: Frank.Nawroth@amedes-group.com

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_24

Effectiveness

Numerous studies are now available as the basis for several meta-analyses in the recent years. Disadvantages of some studies are the heterogeneous collectives and suboptimal outcome parameters (amenorrhea rate or FSH value) instead of AMH value measurement (anti-Müllerian hormone) in the serum pretherapeutically and after a sufficiently long follow-up after the end of therapy. Concern has long been raised that the use of GnRHa in hormone receptor–positive tumours could affect the responsiveness of tumour cells to chemotherapy. However, literature data could not confirm this assumption [4].

Since 2014, various studies investigating parallel chemotherapy/GnRHa administration have shown a significantly lower rate of premature ovarian insufficiency (POI) in different cancer types (breast cancer, ovarian cancer, lymphoma) [5] and in women with breast cancer [6–8]. In one meta-analysis with breast cancer, ovarian cancer and lymphoma patients, POI risk was reduced but reduction did not reach significance [9].

Two meta-analyses did not demonstrate any effect on the clinical and laboratory parameters investigated in breast cancer patients [10, 11].

Two prospective randomized studies [7, 12] and two meta-analyses [6, 8] showed a significantly higher pregnancy rate in the GnRHa group in addition to the above-mentioned positive influence on the POI rate (Table 1; Figs. 1 and 2).

As a result of the studies mentioned above, in 2020, the Breast Commission of the German Gynaecological Oncology Working Group (AGO e.V.) decided to recommend GnRHa for fertility preservation, independent of the hormone receptor [13].

The current Cochrane analysis which included patients with breast cancer, ovarian cancer and Hodgkin's lymphoma confirms the protective effect of GnRHa on the maintenance of ovarian function but calls for further studies into the effects on fertility [14].

Long-Term Fertility-Protective Effect

A criticism of the studies is the short follow-up period and the resulting question of whether a fertility-protective effect of GnRHa would be detectable over several years.

Demeestere et al. [15] studied 67 lymphoma patients (26.2 ± 0.6 years) over a median follow-up of 5.3 (GnRHa group) or 5.6 years (control group). After this time, they found no significant difference in the POI rate or in serum AMH and FSH values.

Lambertini et al. [16], however, confirmed a long-term protective effect on ovarian function in breast cancer patients with a median follow-up of 7.3 years (6.3–8.2 years). The cumulative 5-year incidence of a maintained menstrual period was 72.6% (95%CI, 65.7%–80.3%) in the GnRHa group vs. 64.0% (95%CI, 56.2%–72.8%) in the control group. Adjusted for age, there was a significant difference (Hazard ratio 1.48; 95%CI, 1.12–1.95; $P = 0.006$).

Table 1 Relevant Study Results Between 2014 and 2018

Literature	Design	Results with GnRHa
Del Mastro et al. 2014 [5]	Meta-analysis (patients with breast cancer, ovarian cancer, lymphoma)	– Significant reduction of POI: OR 0.43 (0.22–0.84)
Vitek et al. 2014 [11]	Meta-analysis (patients with breast cancer)	– No significant difference regarding recovery of menstruation: OR 1.47 (0.60–3.62)
Moore et al. 2015 [7]	Prospective-randomized study (patients with breast cancer)	– Significant reduction of POI: OR 0.30 (0.09–0.97) – Significant higher pregnancy rate (21 vs. 11%) – Significant higher disease free ($P = 0.04$) and overall survival ($P = 0.05$)
Elgindy et al. 2015 [10]	Meta-analysis (patients with breast cancer)	– No significant difference regarding recovery of menstruation: RR 1.12 (0.99–1.27) – No significant difference regarding FSH. AMH and AFC (Antral follicle count)
Munhoz et al. 2016 [8]	Meta-analysis (patients with breast cancer)	– Significantly higher rate of eumenorrhea 6 and minimum of 12 months after last chemotherapy: OR 2.41 (1.40–4.15) and 1.85 (1.33–2.59) – Significantly higher pregnancy rate: OR 1.85 (1.02–3.36)
Lambertini et al. 2018 [6]	Meta-analysis (patients with breast cancer)	– Significant reduction of POI: OR 0.38 (0.26–0.57) – Significantly higher pregnancy rate: OR 1.83 (1.06–3.15)
Hickman et al. 2018 [9]	Meta-analysis (patients with breast cancer, ovarian cancer, lymphoma)	– Higher POI rate without GnRHa: OR 1.83 (1.34–2.49. Not significant)
Moore et al. 2019 [12]	Prospective-randomized study (longer follow-up than [7]) (patients with breast cancer)	– Significantly higher pregnancy rate (23.1 vs. 12.2%) – No significant difference in disease-free ($P = 0.09$) and overall survival ($P = 0.06$)

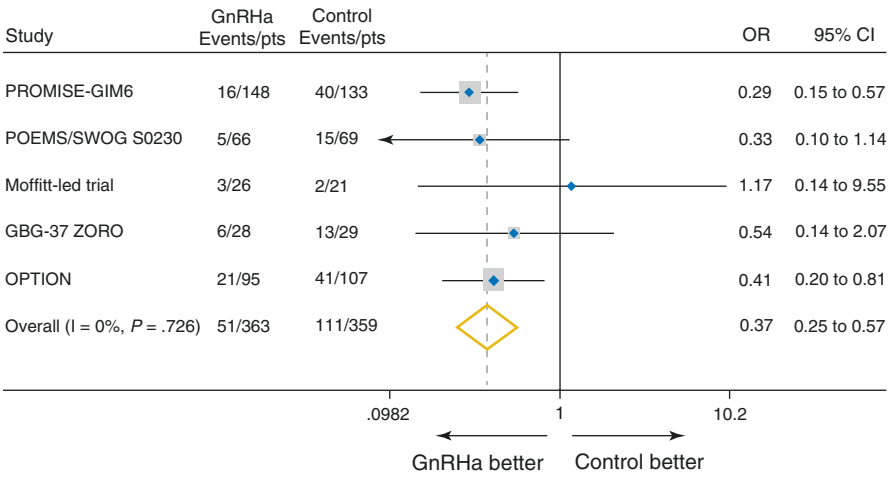


Fig. 1 POI risk of breast cancer patients with and without GnRHa [6]

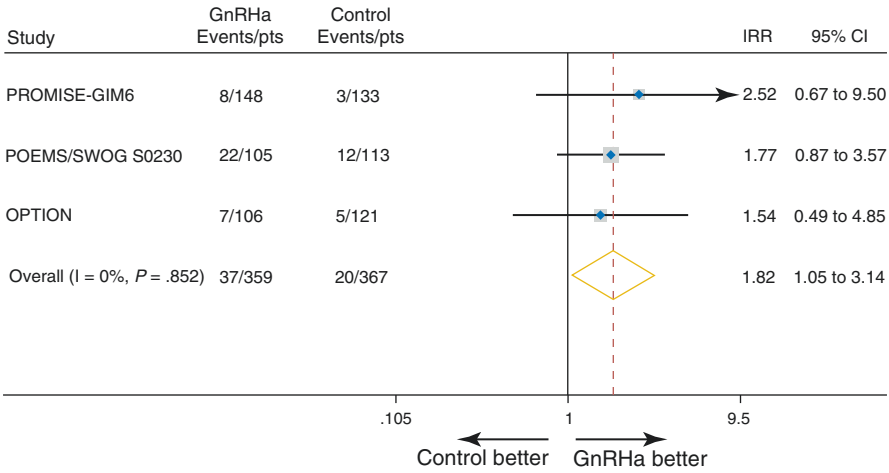


Fig. 2 Pregnancy rate in breast cancer patients with and without GnRHa [6]

Regarding the longevity of the GnRHa protective effect, there is a need for further clarification in the absence of further studies.

Risks (Including Recurrence Rate)

In principle, GnRHa can lead to menopausal symptoms. However, suppression of the ovarian function results anyway during chemotherapy, and such symptoms are possible a few days earlier at the most as a result of the previously administered GnRHa.

The irreversible loss of bone density that can occur with administration for >6 months is normally irrelevant because chemotherapy usually does not exceed this duration.

There is currently no evidence to support the above-mentioned assumption that GnRHa could theoretically impair the efficacy of chemotherapy in hormone receptor–positive tumours [4]. Regan et al. [17] found no negative influence of GnRHa on the disease-free interval in a follow-up of up to 5 years after the end of chemotherapy in breast cancer patients.

Procedure

If there is little time remaining between the consultation and the planned start of chemotherapy, the pituitary (and thus also ovarian) down-regulation could be shortened by additionally combining the first GnRHa dose with a GnRHant for a few days.

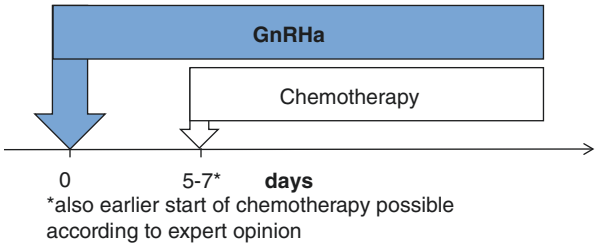


Fig. 3 Application of GnRHa

As already mentioned, it is not known whether the flare-up must be awaited or prevented at all, since the primordial follicles to be protected—as described above—are not gonadotropin sensitive. According to current expert opinion, chemotherapy can also be started more quickly after GnRHa administration.

A depot GnRHa should be injected repeatedly so that the down-regulation lasts about 1–2 weeks beyond the last chemotherapy cycle.

Practical Application

The practical procedure is shown in Fig. 3.

References

1. Xu M, Pavone ME, Woodruff T. Fruitful progress to fertility: preserving oocytes from chemodestruction. Nat Med. 2011;17:1562–3. https://doi.org/10.1038/nm.2595.
2. Lambertini M, Horicks F, Del Mastro L, Partridge AH, Demeestere I. Ovarian protection with gonadotropin-releasing hormone agonists during chemotherapy in cancer patients: from biological evidence to clinical application. Cancer Treat Rev. 2019;72:65–77. https://doi.org/10.1016/j.ctrv.2018.
3. von Wolff M, Kämmerer U, Kollmann Z, Santi A, Dietl J, Frambach T. Combination of gonadotropin-releasing hormone (GnRH) agonists with GnRH antagonists before chemotherapy reduce but does not completely prevent a follicle-stimulating hormone flare-up. Fertil Steril. 2011;95:452–4. https://doi.org/10.1016/j.fertnstert.2010.08.053.
4. Pagani O, Regan MM, Walley BA, Fleming GF, Colleoni M, Láng I, Gomez HL, Tondini C, Burstein HJ, Perez EA, Ciruelos E, Stearns V, Bonnefoi HR, Martino S, Geyer CE Jr, Pinotti G, Puglisi F, Crivellari D, Ruhstaller T, Winer EP, Rabaglio-Poretti M, Maibach R, Ruepp B, Giobbie-Hurder A, Price KN, Bernhard J, Luo W, Ribi K, Viale G, Coates AS, Gelber RD, Goldhirsch A, Francis PA, TEXT and SOFT Investigators; International Breast Cancer Study Group. Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. N Engl J Med. 2014;371:107–18. https://doi.org/10.1056/NEJMoa1404037.
5. Del Mastro L, Ceppi M, Poggio F, Bighin C, Peccatori F, Demeestere I, Levaggi A, Giraudi S, Lambertini M, D'Alonzo A, Canavese G, Pronzato P, Bruzzi P. Gonadotropin-releasing hormone analogues for the prevention of chemotherapy-induced premature ovarian failure in cancer women: systematic review and meta-analysis of randomized trials. Cancer Treat Rev. 2014;40:675–83. https://doi.org/10.1016/j.ctrv.2013.12.001.

6. Lambertini M, Moore HCF, Leonard RCF, Loibl S, Munster P, Bruzzone M, Boni L, Unger JM, Anderson RA, Mehta K, Minton S, Poggio F, Albain KS, Adamson DJA, Gerber B, Cripps A, Bertelli G, Seiler S, Ceppi M, Partridge AH, Del Mastro L. Gonadotropin-releasing hormone agonists during chemotherapy for preservation of ovarian function and fertility in premenopausal patients with early breast cancer: a systematic review and meta-analysis of individual patient-level data. J Clin Oncol. 2018;36:1981–90. https://doi.org/10.1200/JCO.2018.78.0858.
7. Moore HC, Unger JM, Phillips KA, Boyle F, Hitre E, Porter D, Francis PA, Goldstein LJ, Gomez HL, Vallejos CS, Partridge AH, Dakhil SR, Garcia AA, Gralow J, Lombard JM, Forbes JF, Martino S, Barlow WE, Fabian CJ, Minasian L, Meyskens FL Jr, Gelber RD, Hortobagyi GN, Albain KS, POEMS/S0230 Investigators. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy. N Engl J Med. 2015;372:923–32. https://doi.org/10.1056/NEJMoa1413204.
8. Munhoz RR, Pereira AA, Sasse AD, Hoff PM, Traina TA, Hudis CA, Marques RJ. Gonadotropin-releasing hormone agonists for ovarian function preservation in premenopausal women undergoing chemotherapy for early-stage breast cancer: a systematic review and meta-analysis. JAMA Oncol. 2016;2:65–73. https://doi.org/10.1001/jamaoncol.2015.3251.
9. Hickman LC, Llarena NC, Valentine LN, Liu X, Falcone T. Preservation of gonadal function in women undergoing chemotherapy: a systematic review and meta-analysis of the potential role for gonadotropin-releasing hormone agonists. J Assist Reprod Genet. 2018;35:571–81. https://doi.org/10.1007/s10815-018-1128-2.
10. Elgindy E, Sibai H, Abdelghani A, Mostafa M. Protecting ovaries during chemotherapy through gonad suppression: a systematic review and meta-analysis. Obstet Gynecol. 2015;126:187–95. https://doi.org/10.1097/AOG.0000000000000905.
11. Vitek WS, Shayne M, Hoeger K, Han Y, Messing S, Fung C. Gonadotropin-releasing hormone agonists for the preservation of ovarian function among women with breast cancer who did not use tamoxifen after chemotherapy: a systematic review and meta-analysis. Fertil Steril. 2014;102:808–15.e1. https://doi.org/10.1016/j.fertnstert.2014.06.003.
12. Moore HCF, Unger JM, Phillips KA, Boyle F, Hitre E, Moseley A, Porter DJ, Francis PA, Goldstein LJ, Gomez HL, Vallejos CS, Partridge AH, Dakhil SR, Garcia AA, Gralow JR, Lombard JM, Forbes JF, Martino S, Barlow WE, Fabian CJ, Minasian LM, Meyskens FL, Gelber RD, Hortobagyi GN, Albain KS. Final analysis of the prevention of Early Menopause Study (POEMS)/SWOG intergroup S0230. J Natl Cancer Inst. 2019;111:210–3. https://doi.org/10.1093/jnci/djy185.
13. Arbeitsgemeinschaft Gynäkologische Onkologie e.V. (AGO), Kommission Mamma 2020. https://www.ago-online.de/fileadmin/agoonline/downloads/_leitlinien/kommission_mamma/2020/Alle_aktuellen_Empfehlungen_2020.pdf.
14. Chen H, Xiao L, Li J, Cui L, Huang W. Adjuvant gonadotropin-releasing hormone analogues for the prevention of chemotherapy-induced premature ovarian failure in premenopausal women. Cochrane Database Syst Rev. 2019;3:CD008018. https://doi.org/10.1002/14651858.CD008018.pub3.
15. Demeestere I, Brice P, Peccatori FA, Kentos A, Dupuis J, Zachee P, Casasnovas O, Van Den Neste E, Dechene J, De Maertelaer V, Bron D, Englert Y. No evidence for the benefit of gonadotropin-releasing hormone agonist in preserving ovarian function and fertility in lymphoma survivors treated with chemotherapy: final long-term report of a prospective randomized trial. J Clin Oncol. 2016;34:2568–74. https://doi.org/10.1200/JCO.2015.65.8864.
16. Lambertini M, Boni L, Michelotti A, Gamucci T, Scotto T, Gori S, Giordano M, Garrone O, Levaggi A, Poggio F, Giraudi S, Bighin C, Vecchio C, Sertoli MR, Pronzato P, Del Mastro L, GIM Study Group. Ovarian suppression with triptorelin during adjuvant breast cancer chemotherapy and long-term ovarian function, pregnancies, and disease-free survival: a randomized clinical trial. JAMA. 2015;314:2632–40. https://doi.org/10.1001/jama.2015.17291.

17. Regan MM, Walley BA, Francis PA, Fleming GF, Láng I, Gómez HL, Colleoni M, Tondini C, Pinotti G, Salim M, Spazzapan S, Parmar V, Ruhstaller T, Abdi EA, Gelber RD, Coates AS, Goldhirsch A, Pagani O. Concurrent and sequential initiation of ovarian function suppression with chemotherapy in premenopausal women with endocrine-responsive early breast cancer: an exploratory analysis of TEXT and SOFT. Ann Oncol. 2017;28:2225–32. https://doi.org/10.1093/annonc/mdx285.

Ovarian Transposition

Matthias Korell

Introduction

The reason for ovarian transposition is to protect them from planned radiotherapy. This procedure was first described in 1958 in women with cervical cancer [1]. The method was published in 1970 for women undergoing Y-field irradiation for Hodgkin's lymphoma [2].

The objectives were to preserve endocrine ovarian function and to enable conception after oncological treatment. There is no standard terminology in the literature—there is mention of "ovarian transposition", "lateral or medial ovariopexy", "ovarian suspension" or "oophoropexy".

The effects of radiotherapy on ovarian function are considerable. A radiation dose of 2 Gy to the ovaries (LD50) reduces the follicle density by half [3]. The radiation dose for complete elimination of ovarian function in a 30-year-old woman is 16 Gy [4]. Further information on the gonadotoxicity of radiotherapy is given in chapter "Indications for and Against Fertility Preservation".

An indication for transposition can made especially if the ovaries lie directly in the radiation field or lie close to the radiation field, but radiation exposure is still possible (Table 1).

The extent of damage to the ovaries caused by radiotherapy can be quantified by determining the concentration of AMH (anti-Müllerian hormone). AMH can also be used as a marker of the ovarian reserve in childhood [5]. Inhibin and FSH, on the other hand, are less suitable for monitoring progress.

The question of whether a uni- or bilateral ovarian transposition should be performed prior to a radiotherapy can only be decided individually. In addition to the expected gonadotoxicity (taking into account any additionally planned chemotherapy), the possible wish for unilateral transposition plays a role in allowing

M. Korell (✉)
Department of Gynaecology and Obstetrics, Johanna Etienne Hospital, Neuss, Germany
e-mail: m.korell@ak-neuss.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_25

Table 1 Frequent diseases with a possible indication for ovarian transposition when radiotherapy is performed in the pelvis

Hodgkin's lymphoma
Non-Hodgkin's lymphoma
Rectal cancer
Ewing's sarcoma of the pelvis
Cervical cancer
Other indications for pelvic radiation

Table 2 Advantages and disadvantages of ovarian transposition prior to radiotherapy

Advantages
Established procedure
High chance of preserving ovarian function
Can be combined with cryopreservation of ovarian tissue
Can be combined with other surgical procedures (e.g. lymph node dissection)
Disadvantages
Time needed approximately 1 week
Operation necessary (laparoscopy)
Reduced success rate in combination with chemotherapy (possibly also cryopreservation of oocytes and/or ovarian tissue necessary)
Very low chance of spontaneous pregnancies due to tubal dysfunction and especially if simultaneous radiotherapy to the uterus is performed

spontaneous conception via the remaining contralateral side. The advantages and disadvantages of ovarian transposition are summarized in Table 2.

Effectiveness

The results of ovarian transplantation before radiotherapy depend on various factors and are therefore difficult to quantify. In a meta-analysis of 32 publications with a total of 1189 patients, a success rate in terms of preserved ovarian function is given as 70% (17–95%) [6]. However, a publication bias is possible, since many cases or studies with a low success rate are unlikely to have been published [7].

In addition to age, the factor influencing effectiveness is the additional use of chemotherapy, whereby monotherapy as a "radiosensitizer" has a lower impact than combination therapy [8, 9].

Furthermore, the success rate of ovarian transplantation is also determined by the surgical technique. Different techniques such as cranial, lateral, medial and anterior transposition are described [10]. Due to the inhomogeneity of the collectives and the lack of prospective randomized studies, no reliable statement can be made when comparing the different techniques.

However, the importance of the new position of the ovaries has been proven. The decisive factor here is the distance to the radiation field, since approximately 10% of the radiation dose is still effective at a distance of 10 cm from the radiation field

[11]. Despite transposition, radiotherapy to the entire pelvis leads to ovarian insufficiency much more frequently than localized afterloading (35% vs. 6%) [12]. For this reason, the surgeon must consult carefully with the radiotherapists before the planned transposition.

The positioning level is also a relevant prognostic factor. In a multivariate analysis, the height of the transposed ovary with an odds ratio of 11.7 was the most relevant prognostic factor for maintenance of ovarian function. The ovary should lie at least 2 cm above the iliac crest [13]. A safety distance of about 2 cm must also be considered, as the position of the ovaries can change postoperatively [14].

Data on the effectiveness of ovarian transplantation in terms of the chance of later pregnancy and birth is limited, especially since pregnancies do not occur very often after transplantation [15]. This may also be due to the fact that there may no longer be a desire to have children after the end of oncological treatment, or the use of assisted reproductive techniques is not considered [16]. Radiation of the uterus has also been shown to reduce the chances of pregnancy (see chapter "Pregnancy After Chemotherapy and Pelvic Radiotherapy"). Here, a "uterine transposition" in women with anal or rectal carcinoma could possibly reduce the radiation dose for the uterus [17]. Further investigations must be carried out for a more precise evaluation and description of this surgical technique.

Fernandez-Pineda et al. [18] compared women with Hodgkin's lymphoma with pelvic radiation with and without ovarian transposition. Transposition was performed in 49 women but not in 41. Transposition was defined as retro-uterine fixation of the ovaries in the median line. This technique did not lead to a reduction in the POI risk nor to an improved chance of pregnancy. These data indicate a preference for high ovarian transposition. However, this later requires an assisted reproductive technique due to the fallopian tubes being dissected.

Risks

The surgical risks of ovarian transplantation can be classified as low. In most cases, the procedure can be performed by laparoscopy. If an operation is performed for another indication, the ovarian transposition can be performed simultaneously without significantly increasing the complication rate.

Ovarian cysts rarely form postoperatively and are more an expression of impaired ovarian function [6]. In most cases, however, these cysts do not require treatment.

Ovarian metastases seem to occur more frequently in cervical adenocarcinoma (1.7%) than in squamous cell carcinoma (0.5%) [19]. The frequency of metastases at the trocar insertion sites ("port site metastasis") is stated as <1% [6].

Practical Approach

The abdomen is first examined usually via laparoscopic access. It is particularly important to exclude the possibility of intra-abdominal tumour spread. Other indicated interventions such as lymphonodectomy should be performed first [18]. A simultaneously planned cryopreservation of ovarian tissue should also be preferred, and the ovary closed with a suture.

It is often necessary to detach the adnexa from the uterus after visualization of the ipsilateral ureter (Fig. 1a) before detaching the ovary and fallopian tube (Fig. 1b). Stapling devices can ideally be used to avoid coagulation, which could impair the ovarian function. The ovary is marked with titanium clips to localize the ovaries by the radiologist.

The infundibular pedicle is prepared cranially (Fig. 1c) until tension-free fixation of the ovary in the target position is possible. Coagulation and/or torsion of the ovarian vessels must be avoided. The blood flow can be visualized well using the tube, whereas this is not possible with the ovary. The ovary is fixed in the target position, for example with simple interrupted sutures (Fig. 1d).

To avoid intestinal obstruction, the vascular pedicle should also be fixed to the abdominal wall.

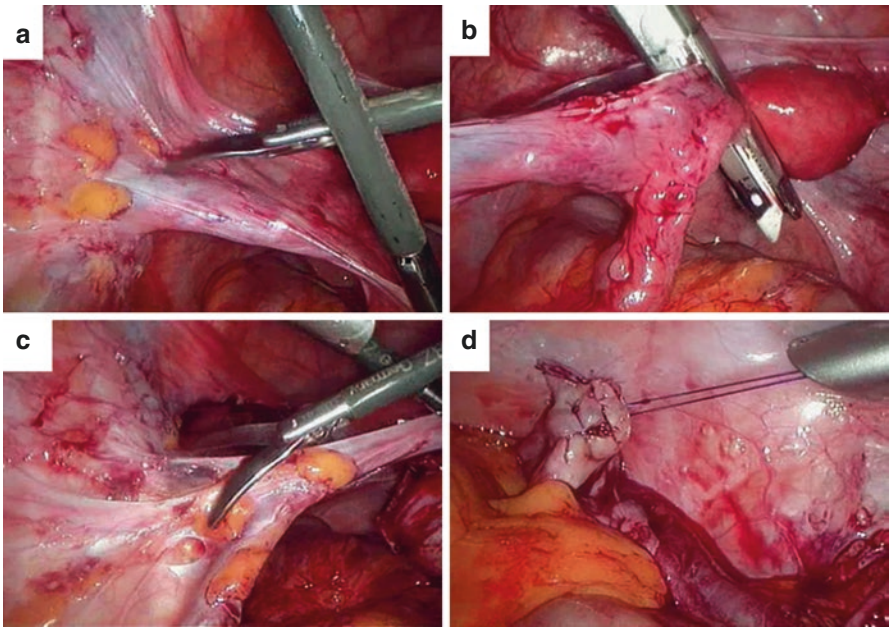


Fig. 1 Pictures of endoscopic transposition with step-by-step subdiaphragmatic relocation of the ovary. (**a**) Retroperitoneal preparation to visualize the ureter and mobilization of the adnexa. (**b**) Detaching of adnexa with a linear cutter. (**c**) Preparation of the cranial ovarian vessels. (**d**) Fixation of adnexa by suture

References

1. McCall ML, Keaty EC, Thompson JD. Conservation of ovarian tissue in the treatment of the carcinoma of the cervix with radical surgery. Am J Obstet Gynecol. 1958;75:590–600.
2. Ray GR, Trueblood HW, Enright LP, Kaplan HS, Nelsen TS. Oophoropexy: a means of preserving ovarian function following pelvic megavoltage radiotherapy for Hodgkin's disease. Radiology. 1970;96:175–80.
3. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod. 2003;18:117–21.
4. Irtan S, Orbach D, Helfre S, Sarnacki S. Ovarian transposition in prepubescent and adolescent girls with cancer. Lancet Oncol. 2013;14:601–8.
5. Brougham MF, Crofton PM, Johnson EJ, Evans N, Anderson RA, Wallace WH. Anti-Müllerian hormone is a marker of gonadotoxicity in pre- and postpubertal girls treated for cancer: a prospective study. J Clin Endocrinol Metab. 2012;97:2059–67.
6. Mossa B, Schimberni M, Di Benedetto L, Mossa S. Ovarian transposition in young women and fertility sparing. Eur Rev Med Pharmacol Sci. 2015;19:3418–25.
7. Kicinski M, Springate DA, Kontopantelis E. Publication bias in meta-analyses from the Cochrane database of systematic reviews. Stat Med. 2015;10(34):2781–93.
8. Roberts J, Ronn R, Tallon N, Holzer H. Fertility preservation in reproductive-age women facing gonadotoxic treatments. Curr Oncol. 2015;22:294–304.
9. Sklar CA, Mertens AC, Mitby P, Whitton J, Stovall M, Kasper C, Mulder J, Green D, Nicholson HS, Yasui Y, Robison LL. Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. J Natl Cancer Inst. 2006;98:890–6.
10. Visvanathan DK, Cutner AS, Cassoni AM, Gaze M, Davies MC. A new technique of laparoscopic ovariopexy before irradiation. Fertil Steril. 2003;79:1204–6.
11. Winarto H, Febia E, Purwoto G, Nuranna L. The need for laparoscopic ovarian transposition in young patients with cervical cancer undergoing radiotherapy. Int J Reprod Med. 2013;2013:173568.
12. Gubbala K, Laios A, Gallos I, Pathiraja P, Haldar K, Ind T. Outcomes of ovarian transposition in gynaecological cancers; a systematic review and meta-analysis. J Ovarian Res. 2014;25:69.
13. Hwang JH, Yoo HJ, Park SH, Lim MC, Seo SS, Kang S, Kim JY, Park SY. Association between the location of transposed ovary and ovarian function in patients with uterine cervical cancer treated with (postoperative or primary) pelvic radiotherapy. Fertil Steril. 2012;97:1387–93.
14. Soda I, Ishiyama H, Ono S, Takenaka K, Arai M, Arai T, Iwase H, Sekiguchi A, Kawakami S, Komori S, Onda T, Hayakawa K. Assessment of transposed ovarian movement: how much of a safety margin should be added during pelvic radiotherapy? J Radiat Res. 2015;56:354–9.
15. Kurt M, Uncu G, Cetintas SK, Kucuk N, Guler S, Ozkan L. Successful spontaneous pregnancy in a patient with rectal carcinoma treated with pelvic radiotherapy and concurrent chemotherapy: the unique role of laparoscopic lateral ovary transposition. Eur J Gynaecol Oncol. 2007;28:408–10.
16. Salih SM, Albayrak S, Seo S, Stewart SL, Bradley K, Kushner DM. Diminished utilization of in vitro fertilization following ovarian transposition in cervical cancer patients. J Reprod Med. 2015;60:345–53.
17. Azaïs H, Canova CE, Vesale E, Simon JM, Canlorbe G, Uzan C. Laparoscopic uterine fixation to spare fertility before pelvic radiation therapy. Fertil Steril. 2018;110:974–5.
18. Fernandez-Pineda I, Davidoff AM, Lu L, Rao BN, Wilson CL, Srivastava DK, Klosky JL, Metzger ML, Krasin MJ, Ness KK, Pui CH, Robison LL, Hudson MM, Sklar CA, Green DM, Chemaitilly W. Impact of ovarian transposition before pelvic irradiation on ovarian function among long-term survivors of childhood Hodgkin lymphoma: a report from the St. Jude Lifetime Cohort Study. Pediatr Blood Cancer. 2018;65:e27232.
19. Sutton GP, Bundy BN, Delgado G, Sevin BU, Creasman WT, Major FJ, Zaino R. Ovarian metastases in stage IB carcinoma of the cervix: a Gynecologic Oncology Group study. Am J Obstet Gynecol. 1992;166:50–3.

Cryopreservation of Sperm and Testicular Tissue

Sabine Kliesch

Introduction

Cryopreservation of semen is the preventive treatment option to enable male adolescents and adults who have a fertility disorder or who are threatened by infertility [1, 2]. According to recent publications, informing oncological patients about the possibility of cryopreservation before a potentially gonadotoxic treatment can still be improved and only reaches 39% of those affected [3]. A corresponding consensus-based German Austrian Swiss guideline (AWMF-S2k) on "Fertility Maintenance in Oncological Diseases" was therefore drawn up under the auspices of the gynaecological, reproductive and urological professional associations in Germany and published in 2018 [4].

However, non-oncological diseases can also be associated with a potentially gonadotoxic or germ cell-reducing (surgical) treatment and should be reason to discuss fertility preservation measures such as the cryopreservation of sperm.

The World Health Organization, WHO, also recommends that men who want to undergo elective vasectomy should be advised on preoperative cryopreservation of sperm [5, 6]. In men who have no sperm in the ejaculate (azoospermia) or cannot ejaculate, there is the possibility of obtaining and cryopreserving sperm from the testicular tissue by surgical scrotal exploration and testicular sperm extraction, ideally microsurgically [2, 7, 8]. In very rare cases, retrograde ejaculation (post-traumatic, post-operative or post-radiogenic) allows the cryopreservation of sperm from urine or after rectal stimulation.

For prepubertal boys or early adolescents whose spermatogenesis is not yet mature, the only experimental option currently available is the removal of testicular

S. Kliesch (✉)
Department of Clinical and Surgical Andrology, Centre of Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany
e-mail: Sabine.Kliesch@ukmuenster.de

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_26

tissue from the immature tissue in which the spermatogonial stem cells rest and can be cryopreserved [9, 10].

In 2012, the Münster working group in Germany led by S. Kliesch and S. Schlatt founded the German "Andropro tect" network, which created the framework and conditions for these measures in Germany [11]. This network currently includes five centres in Germany (another four are in the application phase), which offer this fertility preservation measure to affected children and their parents in cooperation with the Münster Centre of Reproductive Medicine and Andrology.

Prepubertal testicular tissue collection with spermatogonial stem cells was also included in the above-mentioned German, Austrian and Swiss AWMF-S2k guidelines in 2018 [4].

Internationally, the "Nordfertil" network (Nordic Centre for Fertility Preservation) in the Nordic countries was founded in 2013 and the Oncofertility Consortium in the United States in 2006, the latter having been comfortably provided with start-up financing of $20 million. Within the framework of research alliances, intensive work has also been carried out in Europe ("GROWSPERM", 2014–2019) on the further development of the methodology in order to achieve re-fertilization of the affected person from testicular stem cells using in vitro or in vivo procedures at a later point in time [8, 12]. A breakthrough was published in Pittsburgh in 2019: it could be proven for the first time that prepubertal testicular tissue obtained from testicles after transplantation in non-human primates leads to the maturation of seminiferous tubules and mature spermatozoa which, using intra-cytoplasmatic sperm injection, ICSI, resulted in pregnancy and subsequently in the birth of a monkey baby ("Grady", graft-derived baby) [13, 14].

Indication for Cryopreservation of Sperm and Testicular Tissue

The indication for cryopreservation of sperm and testicular tissue extends to all systemic treatments that have potential gonadotoxic effects, as well as to all local treatments that may affect gonadal function directly or via their control mechanisms. In addition, there is a medical need for fertility preservation in surgical procedures that have a long-term adverse effect on sperm deposition (ejaculation and/or erection). In countries where the use of cryopreserved semen samples is also permitted posthumously, cryopreservation is also recommended for men within the scope of a hazardous activity, e.g. military service [2]. In principle, it is possible for every man to create a fertility reserve ("social freezing") [15] (Tables 1 and 2).

Table 1 Advantages and disadvantages of sperm cryopreservation

Advantages of sperm cryopreservation	Disadvantages of sperm cryopreservation
Creation of a cryodepot for later fertility treatment. In principle, cryopreservation is also possible with <100,000 sperm.	The sperm lose up to 50% of their vitality through the process of freezing and later thawing. This means that if the sperm quality is very limited (<100,000 sperm), it must be checked whether the quality after thawing also represents an effective fertility reserve.
The cryodepot can be transferred to the respective place of residence of the owner for fertility treatment, taking into account the legal requirements.	Depending on country-specific regulations, the costs of the procedure must be (proportionately) borne by the patients themselves or are covered by the health insurance companies.
If the semen quality is very good and the semen deposit is large enough, simple methods of fertility treatment, such as intrauterine insemination, may also be used.	The use of cryopreserved sperm cells is only reasonably possible using ICSI therapy for most of those affected.
When cryopreserving sperm from urine or after rectal electrostimulation, the quality of the sperm must be checked carefully after freezing and thawing, since there is often a complete loss of vitality.	Due to the environment, sperm from the urine is only very rarely suitable for cryopreservation. In this case, a testicular tissue removal for sperm extraction can be advantageous.
	Cryopreservation is bound by country-specific legal requirements.

Table 2 Advantages and disadvantages of cryopreservation of testicular tissue

Advantages of testicular tissue cryopreservation	Disadvantages of testicular tissue cryopreservation
In the absence of sperm in the ejaculate (azoospermia), cryopreservation of sperm from testicular tissue is the only option for maintaining fertility. Taking into account the legal framework, microsurgical surgical techniques are currently the accepted standard. If these are not regionally available, there is the possibility of multilocular tissue removal, possibly with the higher risk of bleeding and greater traumatization of the remaining testicular parenchyma. This procedure can be used in late and postpubertal adolescents and in men.	• This is a surgical technique measure which must be carried out in specialized centres. • Due to the intervention, the initiation of oncological treatment may have to be postponed for a few days. • The use of testicular (or epididymal) sperm for fertility treatment is only possible as part of ICSI therapy.
The cryopreservation of immature testicular tissue in prepubertal or early pubertal boys focuses on the freezing of gonadal stem cells using special techniques (DMSO based).	The cryopreservation of immature testicular tissue in prepubertal or early adolescent boys is still experimental, and it cannot currently be guaranteed that the later generation of fertilizable sperm cells can actually take place, even if this has been shown in principle in animal experiments and in vitro.
Sperm can be aspirated microsurgically from the epididymis (MESA) if there is uncorrectable obstruction of the draining seminal ducts. These sperm are more mature and motile than testicular sperm.	The motility of sperm from the epididymis is a problem because they are exposed to cryopreservation without the protective testicular tissue.

Effectiveness and Risks

Effectiveness

Cryopreservation of sperm from the ejaculate is an effective therapy, but it is accompanied by an approximately 50% loss of vital cells. Of the patients presenting for cryopreservation of sperm, 40% have a testicular tumour, followed by patients with leukaemia, lymphoma or sarcoma [2]. Approximately 20% of all tumour patients are azoospermic or unable to ejaculate at the time of the disease. For these patients, surgical sperm extraction by means of testicular sperm extraction, ideally using microsurgical techniques, is the only preventive therapy alternative. This procedure gives 60–70% of these patients the chance to freeze fertilizable sperm [2]. Around 50% of couples using the crypreserved sperm will get a baby (see chapter "Infertility treatment after fertility preservation therapies").

The use of cryopreserved sperm samples is particularly useful for patients with oncological diseases, due to the often young age of the patient when a partnership does not yet exist and often only years after completion of the therapy in the "cured" stage with a time delay. Older studies as well as current investigations show the use of cryopreserved sperm in about 8–11% of patients [1, 16, 17]. Unfortunately, around 12% of patients die as a result of the severity of their illness [16]. A temporal recovery of spermatogenesis (sometimes delayed for years) leads to disintegration of the cryodepots, whereby the percentages can be very variable, and 16% of cryodepots were affected in a meta-analysis [17]. Men who used their cryodepots experienced the birth of at least one child in almost half of the cases, with ICSI therapy showing the highest success rates [16, 17].

Risks

There are no risks for the patient associated with the preventive therapy of sperm cryopreservation. Regarding later offspring, there is no evidence that the use of cryopreserved sperm samples from oncologically or non-oncologically diseased patients is associated with an increased risk of malformation of the offspring. However, there are risks in using ICSI therapy per se, which—according to current knowledge from the problems inherent in couples—is associated with a 1.3-fold higher risk of malformation of the offspring [18]. Genetic counselling prior to initiating ICSI therapy is therefore recommended for all couples and is required by the German, Austrian and Swiss AWMF S2k guideline "Diagnostics and therapy prior to assisted reproductive medical treatment" [19, 20].

There is also no risk of tumour cell transmission when using cryopreserved sperm cells in fertility treatment. This also applies to testicular sperm. Testicular sperm are often obtained from regions adjacent to the testicular tumour, especially in patients with a testicular germ cell tumour. Again, there is no increased risk

from the use of these sperm cells or for the offspring from the therapy procedure per se.

Hereditary components of a disease, such as the familial increased risk of a testicular tumour in male descendants of a testicular tumour patient or e.g. genetic aspects of leukaemia, should be considered in the consultation [20, 21].

Surgical sperm extraction is associated with the potential risk of surgery and the associated anaesthesia. In experienced hands, the risk of subsequent bleeding, infection and wound healing disorders is <5% across all patient groups and must be discussed with the patient individually [2].

A low risk arises during long-term storage due to a possible deterioration in semen quality after thawing, although the data are not clear. Huang et al. [22] described an average sperm survival rate of 85.7% after storage for 0–5 years. After storage for 6–10 years and 11–15 years, the survival rate dropped significantly to 82.1% and 73.9%, respectively. Nevertheless, the success rates of IVF therapies were independent of the length of storage. However, this study was not conducted on patients but on healthy sperm donors. Furthermore, it remains unclear whether the expected decrease in semen quality due to the freezing and thawing process was already included in this analysis or not. Based on the clinical experience of a tertiary centre to date, there has been no significant decrease due to the permanent storage of cryopreserved semen samples in oncological patients [1].

Practical Approach

Collection and Cryopreservation of Semen

Collection of Sperm

The ejaculate for fertility preservation should be collected and stored before an adolescent or adult male undergoes any potentially fertility damaging procedure or exposure. This includes surgical procedures as well as radio- or chemotherapy.

- The testicular volume in the low to subnormal adult range can indicate an already initiated spermatogenesis. Adolescent boys have comparable ejaculate parameters for cryopreservation compared to adult patients regardless of the underlying disease [1, 23, 24].
- The sample is obtained by masturbation. A semen sample is obtained rarely by rectal electrostimulation and this method can be discussed especially in early to late adolescents. Since anaesthesia is required in these cases, and the ability to ejaculate is also a sign of maturity in adolescents during puberty development, testicular sperm cell extraction under anaesthesia should be preferred in these cases, since cryopreservation of stem cells is simultaneously possible when the germinal epithelium has not yet matured [9, 10].

The WHO recommends that when cryopreserving normal semen samples, sufficient samples should be preserved for at least 10 or more inseminations to ensure that there is a good chance of pregnancy [5, 6]. In the case of very frequent limitations in ejaculate quality, both in oncological patients for fertility preservation and in infertility patients, the pooling of several samples has not proven to be useful. On similar lines to the WHO recommendation, the frozen quantity should be enough for 10 or more ICSI treatments [5, 6]. This is guaranteed in most cases with standard sets for ejaculate cryopreservation, which contain 36 straws with 300 μL volume each. If the semen quality is significantly reduced (either very few sperm or extremely few motile or vital sperm), the creation of a second depot should be discussed with the patient and made possible.

Cryopreservation of Semen

Cryopreservation, like sperm storage, is a complex procedure and places high demands on the responsibility and reliability of laboratory staff. The requirements for the cryopreservation of sperm are quite different depending on the length of time. In Germany, for example, official approval is required for the laboratories carrying out the procedure. An HIV and hepatitis test which is no more than 3 months old must also be available. A contract with the patient for the cryopreservation and the later storage of the samples is necessary, as well as a corresponding liability insurance of the responsible laboratory.

The WHO does not make any such specific specifications at international level, but there are recommendations on risk management in the current WHO laboratory manual [5, 6], the main features of which are given below:

The basic requirement for the storage of samples is an alarm system for the detection of liquid nitrogen and low atmospheric oxygen. To reduce the risk of cross-contamination with infectious agents between samples during storage (e.g. transmission of HIV, hepatitis B or C through the cryopreservation containers), storage in the vapour phase of liquid nitrogen is preferred and care is taken to ensure that the cryostraws are securely closed. In the vapour phase for storage, special attention must be paid to the temperature of the samples, which must never exceed −130 °C in order to not damage the sperm.

It is essential to ensure the unique identification of the samples. A secure coding system for labelling the cryotubes and vessels is essential. The corresponding codes are noted in all laboratory data sheets and computer databases relating to the patient. When using aliquots of the cryopreserved sperm, the number of remaining samples is documented in the database accordingly. Ideally, a laboratory employee should only process one sample at a time.

There are various cryopreservation protocols for ejaculate sperm as well as commercially available cryoprotective agents that are carefully mixed into the ejaculate, taking into account appropriate incubation periods. Nowadays, cooling and freezing of the ejaculate are usually done with programmable freezers. These control the supply of liquid nitrogen vapour into the freezing chamber and electronically

document the freezing steps. A common freezing protocol cools the samples by 1.5 °C per minute from 20 °C down to −6 °C and then by 6 °C per minute down to −100 °C. After about 40 min, the temperature of the chamber is then maintained at −100 °C for 30 min until the cryocassettes can be transferred to the gas phase of the liquid nitrogen tank for permanent storage. When sending cryopreserved semen samples, the respective national and international laws for the shipment of liquid nitrogen and human biological material must be observed.

Sperm vitrification methods are also currently being tested, which may offer an advantage, especially when the sperm count is low [25].

Before cryopreserved samples are used, they must be thawed and separated from the cryoprotectant. After thawing at room temperature, the contents are processed for assisted fertilization treatment or prepared for the determination of thawing motility (to test the result of cryopreservation). The cryoprotectant must be washed out by dilution in small volume steps.

Removal and Cryopreservation of Testicular Tissue

Removal of Testicular Tissue

Testicular tissue removal with the aim of testicular sperm extraction (TESE) is performed after opening the scrotal skin and exposing the testicles on both sides under local or general anaesthesia and after appropriate preoperative discussion with the patient. Extraction using microsurgical or microscopically assisted techniques is ideal (micro-TESE, mTESE), alternatively from different areas of the testicle (standard-TESE), whereby preservation of the testicular blood flow and meticulous control of bleeding during the procedure must be ensured. In patients with an obstruction as the cause of azoospermia, multilocular tissue sampling is sufficient. In individual cases, microsurgical epididymal aspiration of seminal fluid (MESA) can also be used (for uncorrectable obstruction). Fine needle aspiration is inferior to open testicular tissue removal with regard to sperm yield and postoperative scarring and is not recommended [8].

In prepubertal boys, spermatogenesis has not yet been initiated, and there is only the experimental option of removing spermatogonial stem cells (SSC) using testicular biopsy [9, 10].

When taking testicular tissue for the cryopreservation of spermatogonial stem cells from prepubertal boys or early adolescents, the open biopsy is preferably performed only on one testicle. Since this procedure is experimental, only limited experience is available. For the German network Andropotect, which was founded at the Centre of Reproductive Medicine and Andrology of the University Hospital in Münster in Germany, the collection of three biopsies of approximately the size of a rice grain from one testis is recommended after consent has been obtained. The three biopsies are processed differently (see below). The Andropotect network is currently being set up with the aim of enabling cryopreservation and analysis of

samples from the affected patients for all paediatric oncology and paediatric urology centres. Information can be requested under andrologie@ukmuenster.de. International initiatives can also be found in the United States, the Scandinavian countries (Nordfertil), France, the Netherlands and England.

Cryopreservation of Pre-Pubertal Testicular Tissue (e.g. Androprotect, Münster, Germany)

Within the framework of Androprotect, one of the three tissue samples taken is fixed for (immuno-) histological processing to check for the presence of stem cells. Two samples are prepared for DMSO-based cryopreservation according to protocols for immature testicular tissue, cryopreserved and stored in the gas phase of liquid nitrogen [12, 26]. One sample is stored for the patient, one is used for research. A corresponding ethics vote is available. Three basic strategies are pursued here within the framework of an international research network: (I.) ectopic or orthotopic grafting or xenografting of tissue; (II.) retransplantation of SSC; (III.) in vitro spermatogenesis [11, 27–30]. The Androprotect network currently comprises five centres in Germany, four further centres are in the application phase (ethics vote, cooperation agreements) and the samples taken are sent to the CeRA in Münster, Germany for further analysis, preparation and storage. The accompanying research connected with the network and carried out at CeRA in recent years has provided valuable insights into the prepubertal human germinal epithelium [12, 31–33]. Information on Androprotect can be requested under andrologie@ukmuenster.de.

Cryopreservation of Post-Pubertal Testicular Tissue

The cryopreservation of testicular tissue with testicular sperm varies slightly from laboratory to laboratory. The cryopreservation of sperm in testicular tissue composites is probably preferable to the cryopreservation of isolated sperm after mechanical or enzymatic preparation, especially if the spermatogenesis is only focally preserved, since the tissue composite provides better protection for the sperm during the cryo- and thawing process if the quality of the testicular tissue samples is very limited. Just like the cryopreservation of ejaculate sperm, cryopreservation is carried out after the addition of commercially available cryoprotective agents. The freezing process is carried out step by step with a programmable freezer (see above). The conditions, safety guidelines and requirements for the cryopreservation of post-pubertal testicular tissue correspond to those for ejaculate sperm.

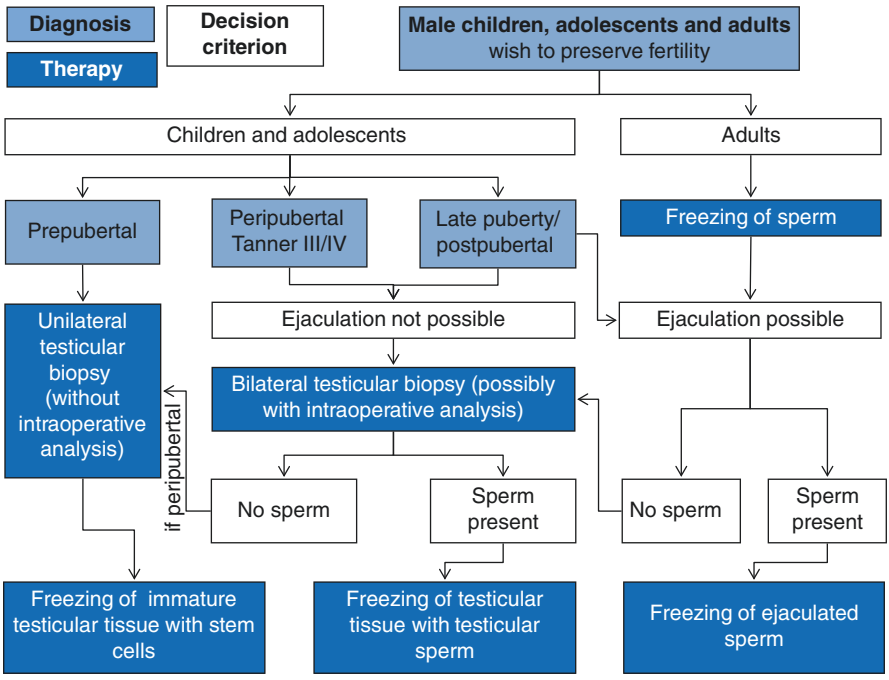


Fig. 1 Algorithm for fertility preservation in boys, adolescents and male adults

Practical Approach

Patients should attend an andrological or reproductive medicine centre with an appropriate laboratory as early as possible for the cryopreservation of sperm and testicular tissue to allow the greatest possible time window for the implementation of fertility preservation measures [16]. In the case of (micro-)TESE, cooperation with a urological-andrological facility is required which meets the infrastructural requirements and offers the surgical procedure accordingly. For the cryopreservation of gonadal stem cells, cooperation with highly specialized centres in Europe is required. For the German-speaking countries, the Centre of Reproductive Medicine and Andrology, University Hospital Münster, is available as a contact partner [10–12].

Figure 1 shows the systematic procedure for carrying out cryopreservation of sperm and testicular tissue in the patient groups.

References

1. Kliesch S, Behre HM, Nieschlag E. Cryopreservation of semen from adolescent patients with malignancies. Med Pediatr Oncol. 1996;26:20–7. https://doi.org/10.1002/(SICI)1096-911X(199601)26:1<20::AID-MPO3>3.0.CO;2-X.
2. Kliesch S, Kamischke A, Cooper TG, Nieschlag E. Cryopreservation of human spermatozoa. In: Nieschlag E, Behre HM, Nieschlag S, editors. Andrology: Male Reproductive Health and Dysfunction. 3rd ed. Berlin, Heidelberg: Springer; 2010. p. 505–20.
3. Nangia AK, Krieg SA, Kim SS. Clinical guidelines for sperm cryopreservation in cancer patients. Fertil Steril. 2013;100:1203–9. https://doi.org/10.1016/j.fertnstert.2013.08.054.
4. Deutsche Gesellschaft für Gynäkologie und Geburtshilfe (DGGG), Deutsche Gesellschaft für Reproduktionsmedizin (DGRM), Deutsche Gesellschaft für Urologie (DGU). Leitlinie: Fertilitätserhaltung bei onkologischen Therapien. Level S2k, AWMF Register Nr. 015/082, November 2017. http://www.awmf.org/leitlinien/detail/ll/015-082.html.
5. World Health Organization, editor. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization; 2010.
6. Nieschlag E, Schlatt S, Behre HM, Kliesch S, unter Mitarbeit von Bongers R, Gottardo F, et al. WHO-Laborhandbuch zur Untersuchung und Aufarbeitung des menschlichen Ejakulates (Übersetzung). 5th ed. Berlin: Springer-Verlag; 2012.
7. Colpi GM, Colpi EM, Piediferro G, Giacchetta D, Gazzano G, Castiglioni FM, et al. Microsurgical TESE versus conventional TESE for ICSI in non-obstructive azoospermia: a randomized controlled study. Reprod Biomed Online. 2009;18:315–9. https://doi.org/10.1016/S1472-6483(10)60087-9.
8. Jungwirth A, Diemer T, Dohle GR, Giwercman A, Kopa Z, Krausz C, Tournaye H. Guidelines on Male Infertility. European Association of Urology, 2015. http://uroweb.org/wp-content/uploads/EAU-Guidelines-Male-Infertility-20151.pdf.
9. Picton HM, Wyns C, Anderson RA, Goossens E, Jahnukainen K, Kliesch S, ESHRE Task Force on Fertility Preservation in Severe Diseases, et al. A European perspective on testicular tissue cryopreservation for fertility preservation in prepubertal and adolescent boys. Hum Reprod. 2015;30:2463–75. https://doi.org/10.1093/humrep/dev190.
10. Kliesch S. Androprotect und Perspektiven der Fertilitätstherapie. Urologe. 2016;55:898–903. https://doi.org/10.1007/s00120-016-0161-y.
11. Schlatt S, Kliesch S. Fertilitätsprotektion bei Männern – Mehr als nur Spermienkryokonservierung. Gynäkol Endokrinol. 2012;10:91–7. https://doi.org/10.1007/s10304-011-0455-5.
12. Sharma S, Wistuba J, Pock T, Schlatt S, Neuhaus N. Spermatogonial stem cells: updates from specification to clinical relevance. Hum Reprod Update. 2019;25:275–97. https://doi.org/10.1093/humupd/dmz006.
13. Fayomi AP, Peters K, Sukhwani M, Valli-Pulask H, Shetty G, Meistrich ML, et al. Autologous grafting of cryopreserved prepubertal rhesus testis produces sperm and offspring. Science. 2019;363:1314. https://doi.org/10.1126/science.aav2914.
14. Neuhaus N, Schlatt S. Stem-cell based options to preserve male fertility. Science. 2019;363:1283–4. https://doi.org/10.1126/science.aaw6927.
15. Gromoll J, Tüttelmann F, Kliesch S. "Social freezing" – die männliche Seite. Urologe. 2016;55:58–62. https://doi.org/10.1007/s00120-015-3943-8.
16. Muller I, Oude Ophuis RJ, Broekmans FJ, Lock TM. Semen cryopreservation and usage rate for assisted reproductive technology in 898 men with cancer. Reprod Biomed Online. 2016;32:147–53. https://doi.org/10.1016/j.rbmo.2015.11.005.
17. Ferrari S, Paffoni A, Filippi F, Busnelli A, Vegetti W, Somigliana E. Sperm cryopreservation and reproductive outcome in male cancer patients: a systematic review. Reprod Biomed Online. 2016;33:29–38. https://doi.org/10.1016/j.rbmo.2016.04.002.
18. von Wolff M, Haaf T. In vitro fertilization technology and child health – risks, mechanisms and possible consequences. Dtsch Arztebl Int. 2020;117:23–30. https://doi.org/10.3238/arztebl.2020.0023.

19. Bundesärztekammer. Richtlinie zur Entnahme und Übertragung von menschlichen Keimzellen im Rahmen der assistierten Reproduktion. Dtsch Ärztebl. 2018;115:A-1096/B-922/C-918. https://doi.org/10.3238/arztebl.2018.Rili_assReproduktion_2018.
20. Al-Jebari Y, Rylander L, Stahl O, Giwercman A. Risk of congenital malformations in children born before paternal cancer. JNCI Cancer Spectr. 2018;2:pky027. https://doi.org/10.1093/jncics/pky027.
21. Al-Jebari Y, Glimelius I, Berglund Nord C, Cohn-Cedermark G, Stahl O, Tandstad T, et al. Cancer therapy and risk of congenital malformations in children fathered by men treated for testicular germ-cell cancer: a nationwide register study. PLoS Med. 2019;16:e1002816. https://doi.org/10.1371/journal.pmed.1002816.
22. Huang C, Lei L, Wu HL, Gan RX, Yuan XB, Fan LQ, et al. Long-term cryostorage of semen in a human sperm bank does not affect clinical outcomes. Fertil Steril. 2019;112:663–9.e1. https://doi.org/10.1016/j.fertnstert.2019.06.008.
23. Bahadur G, Ling KL, Hart R, Ralph D, Riley V, Wafa R, et al. Semen production in adolescent cancer patients. Hum Reprod. 2002;17:2654–6. https://doi.org/10.1093/humrep/17.10.2654.
24. Kamischke A, Jürgens H, Hertle L, Berdel WE, Nieschlag E. Cryopreservation of sperm from adolescents and adults with malignancies. J Androl. 2004;25:586–92. https://doi.org/10.1002/j.1939-4640.2004.tb02829.x.
25. Berkovitz A, Miller N, Silberman M, Belenky M, Itsykson P. A novel solution for freezing small numbers of spermatozoa using a sperm vitrification device. Hum Reprod. 2018;33:1975–83. https://doi.org/10.1093/humrep/dey304.
26. Jahnukainen K, Ehmcke J, Hou M, Schlatt S. Testicular function and fertility preservation in male cancer patients. Best Pract Res Clin Endocrinol Metab. 2011;25:287–302. https://doi.org/10.1016/j.beem.2010.09.007.
27. Schlatt S, Ehmcke J, Jahnukainen K. Testicular stem cells for fertility preservation: preclinical studies on male germ cell transplantation and testicular grafting. Pediatr Blood Cancer. 2009;53:274–80. https://doi.org/10.1002/pbc.22002.
28. Jahnukainen K, Ehmcke J, Nurmio M, Schlatt S. Autologous ectopic grafting of cryopreserved testicular tissue preserves the fertility of prepubescent monkeys that receive sterilizing cytotoxic therapy. Cancer Res. 2012;72:5174–8. https://doi.org/10.1158/0008-5472.CAN-12-1317.
29. Stukenborg JB, Schlatt S, Simoni M, Yeung CH, Elhija MA, Luetjens CM, et al. New horizons for in vitro spermatogenesis? An update on novel three-dimensional culture systems as tools for meiotic and post-meiotic differentiation of testicular germ cells. Mol Hum Reprod. 2009;15:521–9. https://doi.org/10.1093/molehr/gap052.
30. Stukenborg JB, Alves-Lopes JP, Kurekt M, Albalushi H, Reda A, Keros V, et al. Spermatogonial quantity in human prepubertal testicular tissue collected for fertility preservation prior to potentially sterilizing therapy. Hum Reprod. 2018;33:1677–83. https://doi.org/10.1093/humrep/dey240.
31. Neuhaus N, Yoon J, Terwort N, Kliesch S, Seggewiss J, Huge A, et al. Single-cell gene expression analysis reveals diversity among human spermatogonia. Mol Hum Reprod. 2017;23:79–90. https://doi.org/10.1093/molehr/gaw079.
32. Heckmann L, Langenstroth-Röwer D, Pock T, Wistuba J, Stukenborg JB, Zitzmann M, et al. A diagnostic germ cell score for immature testicular tissue at risk of germ cell loss. Hum Reprod. 2018;33:636–45. https://doi.org/10.1093/humrep/dey025.
33. Mincheva M, Sandhowe-Klaverkamp R, Wistuba J, Redmann K, Stukenborg JB, Kliesch S, et al. Reassembly of adult human testicular cells: can testis cord-like structures be created in vitro? Mol Hum Reprod. 2018;24:55–63. https://doi.org/10.1093/molehr/gax063.

Further Fertility Preservation Techniques

Ralf Dittrich and Michael von Wolff

In Vitro Maturation

In vitro maturation (IVM) refers to the removal and maturation of immature oocytes (germinal vesicle stage or metaphase I) after being obtained by transvaginal or aspiration of follicles from removed ovarian tissue.

This technique was originally developed to avoid high-dose gonadotrophin stimulation and therefore the risk of ovarian overstimulation, especially in women with polycystic ovaries. After the turn of the millennium, this technique was routinely used in many centres, especially in women with polycystic ovary syndrome (PCOS). Initial studies showed that the health risk for children after IVM is not noticeably limited [1]. The reported chances of success were relatively high in some centres, especially in women with PCOS.

However, if IVM is performed in women as a fertility preservation measure, these are not usually women with PCOS. Correspondingly, the effectiveness of IVM is significantly lower in these women.

R. Dittrich (✉)
University Hospital Erlangen, OB/GYN, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany
e-mail: Ralf.Dittrich@uk-erlangen.de

M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_27

IVM of Oocytes Collected from Ovaries

Grynberg et al. [2] performed IVM in 248 women with breast cancer (31.5 ± 0.3 years) to avoid ovarian stimulation. The results were similar for oocyte collection in the follicular and luteal phase. Aspiration in the follicular phase led to 6.2 oocytes being cryopreserved, and 6.8 oocytes in the luteal phase. Considering that high-dose gonadotrophin stimulation in breast cancer patients can yield approx. 13 mature oocytes [3, 4], IVM is significantly less effective in terms of oocyte count.

In addition, the pregnancy potential of oocytes after cryopreservation is significantly lower. Cao et al. [5] found that only 13% of the in vitro matured, cryopreserved and then fertilized oocytes developed into embryos, compared to 33% without prior cryopreservation. Rösner et al. [6] reported only two births after 32 thawing cycles in 61 cases wherein vitro mature oocytes had been cryopreserved.

IVM is therefore not very effective. It should not be performed as the sole fertility preservation measure and only in specialized centres in women with a high antral follicle count (AFC), where <2 weeks are available for high-dose gonadotropin stimulation (see chapter "Ovarian Stimulation to Collect Oocytes").

IVM of Oocytes Collected from Ovarian Tissue Before Cryopreservation

Alternatively, IVM can be used to aspirate oocytes from follicles in ovarian tissue prior to cryopreservation.

Huang et al. [7] reported on four women (21–38 years), where a hemiovariectomy was performed in three cases and a total ovariectomy in one. A total of 11 oocytes were aspirated from the tissue taken from the 4 women, 8 of which could be matured and vitrified. Uzelac et al. [8] reported on a 23-year-old female patient from whom 10 immature oocytes were removed after unilateral ovariectomy and 4 of which were matured. However, cryopreservation only took place after the oocytes had been fertilized. Two embryos were later transferred, from which a pregnancy with subsequent birth developed. Abir et al. [9] performed this technique on ovarian tissue in 42 girls and women aged 2–18 years. Three hundred and ninety-five oocytes were obtained and 121 of these were cryopreserved, which corresponds to an average of just under 3 oocytes per girl/woman. It should be noted that in addition to the significantly lower developmental potential after cryopreservation, the oocyte quality also seems to be lower in peripubertal women [10].

These and other case reports show that only a few oocytes can be obtained from biopsied ovarian tissue. If one also considers the low blastocyst-, developmental- and birth rates mentioned above [5, 6], it becomes obvious that this procedure is not very effective and should therefore only be performed in patients with a high AFC and in specialized centres.

Drug Therapy Approaches

Immunomodulator AS101

The immunomodulator AS101 was first described in 1987 by an Israeli working group [11]. AS101 (ammonium trichloro (dioxoethylene-o,o') tellurate) inhibits the anti-inflammatory cytokine IL-10, thereby activating the so-called Akt pathway. In human clinical studies, Akt reduces the hematopoietic side effects of chemotherapy without reducing its effectiveness. In addition, AS101 appears to have an anti-tumour effect [12].

Carmely et al. [13] administered cyclophosphamide intraperitoneally to male mice and gave some animals additional AS101. The administration of AS101 significantly reduced damage to the seminiferous tubules and the DNA fragmentation of the sperm. Mating of male mice with untreated female mice resulted in a higher number and weight of offspring in the group of AS101-treated animals.

Kalich-Philosoph et al. [14] examined the effects of cyclophosphamide with and without AS101 on the follicle pool of mice. They showed that cyclophosphamide induces growth of the primordial follicles, which leads to a kind of "burn out" of the follicle pool and thus to cyclophosphamide-induced ovarian failure. Concomitant treatment with AS101 in mice reduced cyclophosphamide-induced activation of the primordial follicles, resulting in less reduction of the ovarian reserve by chemotherapy.

The fertility-protective effect of AS101 was confirmed in mice in another study from 2017 [15].

Apoptosis Inhibitors Ceramide-I Phosphates, CIP

Numerous studies have shown that chemotherapeutic drugs induce apoptosis and dysregulate angiogenesis. Sphingolipids are polar lipids and components of the cell membrane and, among other things, are involved in the regulation of apoptosis and angiogenesis. One of the bioactive sphingolipid metabolites is sphingosine I-phosphate (CIP). It is assumed that CIP could act as a fertility-preserving agent.

Pascuali et al. [16] administered intraperitoneal cyclophosphamide to mice and gave additional CIP to some. The administration of CIP significantly reduced cyclophosphamide-induced apoptosis, improved stromal vascular function and reduced the decrease in ovarian reserve.

Anti-Müllerian Hormone (AMH)

AMH is predominantly formed by growing follicles, such as secondary follicles, and inhibits the recruitment of primordial follicles. This prevents premature loss of the ovarian reserve. It is believed that chemotherapeutic agents such as cyclophosphamide and cisplatin damage the growing follicles, causing AMH secretion to decrease. Consequently, there is a strong recruitment of primordial follicles and the ovarian reserve therefore decreases. This is known as the "burn out effect".

The concept of administering AMH during chemotherapy is derived from these effects to absorb the chemotherapy-induced drop in AMH.

Experiments where AMH was administered intraperitoneally to mice have confirmed that AMH can compensate for the cyclophosphamide-induced loss of the ovarian reserve [17, 18].

Xenotransplantation of Ovarian Tissue

Xenotransplantation is the transplantation of previously cryopreserved ovarian tissue into other species, mostly immunodeficient mice. The aim is to generate oocytes in other species so that the oocytes can be matured in vitro, fertilized and transferred to females. Xenotransplantation could be used especially in diseases where autologous ovarian tissue transplantation (see chapter "Transplantation of Ovarian Tissue") is contraindicated due to the high risk of contaminating the tissue with tumour cells, e.g. in the case of leukaemia.

Dittrich et al. [19] comprehensively reflected on xenotransplantation in 2015 and evaluated its usefulness as a fertility-protective measure.

As early as 2002, evidence was provided that xenotransplantation is basically possible. Snow et al. [20] transplanted ovarian tissue under the renal capsule of rats. Oocytes developed which led to successful embryo transfers after IVM and fertilization. Studies have also been carried out since 2003, among others by Lotz et al. [21], in which human ovarian tissue was transplanted subcutaneously or intramuscularly into the renal capsule of immunodeficient mice (severe combined immunodeficiency (SCID) mice). Several dozen oocytes could be generated. Most of the mature oocytes (metaphase II), however, originated from smaller follicles than in the human system. For ethical reasons, the oocytes were not fertilized or transferred, therefore there is no proof that xenotransplantation could also be successful in the human system. One objection against xenotransplantation is the transfer of zoonoses, for which the risk seems to be low [22]. On the other hand, ethical concerns have been raised. To our knowledge, however, a comprehensive evaluation of this technique by ethicists has not yet been performed.

In Vitro Growth

In vitro growth (IvG) refers here to the cultivation of oocytes in tissue or isolated follicles to obtain immature oocytes for subsequent IVM. IvG could be used particularly in women in whom ovarian tissue was cryopreserved prior to cytotoxic disease, but where autologous ovarian tissue transplantation (Chapter "Transplantation of Ovarian Tissue") is contraindicated due to the high risk of contamination of the tissue with tumour cells, e.g. in the case of leukaemia.

Telfer and Zelinski [23] carried out a comprehensive investigation of IvG. The main problem lies in the long development time of the follicles from the preantral to the pre-ovulatory stage, which is about 90 days in the human system. It is assumed that a three-stage culture system is necessary.

First the development of the primordial follicles is induced in the tissue, the follicles are then isolated, cultivated and finally immature oocytes are extracted from the follicles and matured in vitro. All individual steps were successfully performed in the human system and a coupling of all three steps was first published as a "proof of concept" by McLauglin et al. at the beginning of 2018 [24]. They cultivated human ovarian tissue from ten women, extracted 87 secondary follicles from them and allowed them to mature further. They then incubated cumulus–oocyte complexes obtained from these follicles until a total of nine oocytes showed a polar body and a metaphase II spindle as in an ovulated oocyte. Morphologically, these IvG oocytes have a large polar body. Their developmental potential is still unknown.

Artificial Ovary

Due to the many technical hurdles that still need to be overcome in IvG, a Brussels working group is taking a different approach. Primordial follicles are isolated from ovarian tissue and fixed in a fibrin matrix, creating a kind of artificial ovarian tissue.

This matrix can then be transplanted orthotopically to allow the follicles to mature in vivo. When transplanting human preantral follicles embedded in alginate, a few antral follicles could be identified after 1 week when transplanted into mice [25]. Since then, there have been several alternative approaches that move away from encapsulating the isolated follicles because of the resulting limitations in terms of space for vascularization/supply, follicular growth and ovulation. The development is moving towards replicating the natural morphology of the ovarian supporting framework, where mechanical properties also play an important role. Laronda et al. [26] developed an artificial ovary whose matrix consists of a gelatine hydrogel with pores for vascularization, follicular growth and ovulation, which was produced by 3D printing. With this approach, they obtained offspring in a mouse model.

Chiti et al. [27] emphasized in a 2018 publication that it is important to orientate towards the natural fibrillar structure of the supporting framework in the ovarian tissue. Liverani et al. [28] considered this while at the same time further developing the material properties of the matrix fibres of the artificial ovary. They used the technique of electrospinning to combine a synthetic polymer, which is widely used in regenerative medicine because of its good mechanical properties and slow degradation, with biocompatible gelatine. The aim is to prolong the possible transplantation time of the artificial ovary while maintaining biocompatibility and thus to adapt it to the duration of human follicle maturation. The latter lasts significantly longer (approx. 90 days) than in the mouse, whose follicles mature after 10–12 days. Liverani et al. [28] therefore worked with pig follicles whose size and maturation period at 40–50 days lie between the mouse model used so far and human conditions.

A transplantation in the human system has not yet been performed. Theoretically, when isolating follicles in leukaemia patients, tumour cells could also be transferred into the matrix. However, various studies show that the risk of this happening seems to be very low [29].

Oocytes from Germ Line Stem Cells or Germline Progenitor Cells Inside the Ovary?

Contrary to the assumption that the number of oocytes in the ovary is fixed from birth and that no new follicles and oocytes are formed, some publications describe the presence of cells that simultaneously have markers of germ line and stem cells. These cells have been detected in various species, including the human ovary, and have been described by some authors as germline stem cells and by others as unipotent ovarian germline precursor cells. Nevertheless, the presence of such cells in the human ovary remains controversial, as some research groups have not been able to reproduce the detection of these cells. There is also disagreement about the function of such potential stem or progenitor cells and clear evidence in the human system is still lacking. It is possible that new oocytes can actually be produced from these cells. So far, it has at least been possible to generate oocyte-like cells—in vitro and using xenotransplantation—from potential stem or progenitor cells of the human ovary. The developmental capacity of these oocyte-like cells is not yet known [30].

If there are germline stem cells or progenitor cells in the ovary and it is possible to produce new oocytes from them, this would be a further option for restoring fertility not only after gonadotoxic treatment, but also for a much larger group of patients, e.g. with premature ovarian insufficiency (POI).

Uterus Transplantation

There is now also the possibility of uterine transplantation. According to the scientific literature, 30 transplantations have been performed to date after the first birth following uterine transplantation by Mats Brännström's Swedish group [31]. However, Brännström et al. assume that twice as many transplantations have been performed. More than ten children have now been born, in one case even after transplantation of the uterus of a deceased woman [31]. The delivery of two children after uterine transplantation due to hysterectomy for cervical cancer has also been published [32].

Nevertheless, the method is still considered to be experimental (Table 1).

Table 1 Further fertility-preserving techniques

Technique	Rationale	Effectiveness in animal model	Effectiveness in humans	Clinical trials	Already usable in humans
In vitro maturation (IVM)	1. Removal of immature oocytes from the ovary to prevent ovarian stimulation 2. Removal of immature oocytes from the ovary prior to cryopreservation of ovarian tissue	Already performed in animals	Established in humans; effectiveness low	Already used by some centres	Yes
Drug therapy options (AS101, CIP, AMH)	Inhibition of chemotherapy-induced reduction of ovarian reserve by: 1. Activating the AKT pathway (AS101) 2. Inhibition of apoptosis (CIP) 3. Inhibition of primordial follicle recruitment (AMH)	Reduction of gonadic toxicity in mice	Not yet proven	No	No

(continued)

Table 1 (continued)

Technique	Rationale	Effectiveness in animal model	Effectiveness in humans	Clinical trials	Already usable in humans
Xenotransplantation	Transplantation of ovarian tissue into immunodeficient animals to generate oocytes, particularly in diseases with a high risk of malignant cells in ovarian tissue	Offspring in mice and rats	Oocytes obtained after transplantation into SCID mice; embryos not yet generated	Not known	No
In vitro growth (IvG)	Cultivation of ovarian tissue or isolated follicles to generate oocytes, particularly in diseases with a high risk of malignant cells in ovarian tissue	Offspring in mice	Development of embryos from primates in mice; development of human germinal vesicles in mice	Not known	No
"Artificial" ovary	Isolation of preantral follicles from ovarian tissue, fixation in a matrix and orthotopic transplantation of the matrix; particularly for diseases with a high risk of malignant cells in ovarian tissue	Development of human follicles in mice; offspring in mice	Not yet applied in humans	Not known	No
Oocytes from germline stem cells/progenitor cells?	Development of oocytes from germline stem cells/ progenitor cells	Offspring in mice	Development of oocyte-like cells from potential germline stem cells/progenitor cells	Not known	No
Uterine transplantation	Transplantation of the uterus after hysterectomy	Yes	>30 transplantations performed with at least ten children; only one transplantation after malignancy with birth (cervical carcinoma)	Yes	Yes

References

1. Roesner S, von Wolff M, Elsaesser M, Roesner K, Reuner G, Pietz J, Bruckner T, Strowitzki T. Two-year development of children conceived by IVM: a prospective controlled single-blinded study. Hum Reprod. 2017;32:1341–50. https://doi.org/10.1093/humrep/dex068.
2. Grynberg M, Poulain M, le Parco S, Sifer C, Fanchin R, Frydman N. Similar in vitro maturation rates of oocytes retrieved during the follicular or luteal phase offer flexible options for urgent fertility preservation in breast cancer patients. Hum Reprod. 2016;31:623–9. https://doi.org/10.1093/humrep/dev325.
3. Quinn MM, Cakmak H, Letourneau JM, Cedars MI, Rosen MP. Response to ovarian stimulation is not impacted by a breast cancer diagnosis. Hum Reprod. 2017;32:568–74. https://doi.org/10.1093/humrep/dew355.
4. von Wolff M, Bruckner T, Strowitzki T, Germeyer A. Fertility preservation: ovarian response to freeze oocytes is not affected by different malignant diseases-an analysis of 992 stimulations. J Assist Reprod Genet. 2018;35:1713–9. https://doi.org/10.1007/s10815-018-1227-0.
5. Cao YX, Chian RC. Fertility preservation with immature and in vitro matured oocytes. Semin Reprod Med. 2009;27:456–64. https://doi.org/10.1055/s-0029-1241055.
6. Roesner S, Von Wolff M, Eberhardt I, Beuter-Winkler P, Toth B, Strowitzki T. In vitro maturation: a five-year experience. Acta Obstet Gynecol Scand. 2012;91:22–7. https://doi.org/10.1111/j.1600-0412.2011.01299.
7. Huang JY, Tulandi T, Holzer H, Tan SL, Chian RC. Combining ovarian tissue cryobanking with retrieval of immature oocytes followed by in vitro maturation and vitrification: an additional strategy of fertility preservation. Fertil Steril. 2008;89:567–72.
8. Uzelac PS, Delaney AA, Christensen GL, Bohler HC, Nakajima ST. Live birth following in vitro maturation of oocytes retrieved from extracorporeal ovarian tissue aspiration and embryo cryopreservation for 5 years. Fertil Steril. 2015;104:1259–60. https://doi.org/10.1016/j.fertnstert.2015.07.1148.
9. Abir R, Ben-Aharon I, Garor R, Yaniv I, Ash S, Stemmer SM, Ben-Haroush A, Freud E, Kravarusic D, Sapir O, Fisch B. 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. 2016;31:750–62. https://doi.org/10.1093/humrep/dew007.
10. Duncan FE. Egg quality during the pubertal transition - is youth all it's cracked up to be? Front Endocrinol (Lausanne). 2017;8:226. https://doi.org/10.3389/fendo.2017.00226.
11. Sredni B, Caspi RR, Klein A, Kalechman Y, Danziger Y, Ben Ya'akov M, Tamari T, Shalit F, Albeck M. A new immunomodulating compound (AS-101) with potential therapeutic application. Nature. 1987;330:173–6.
12. Sredni B. Immunomodulating tellurium compounds as anti-cancer agents. Semin Cancer Biol. 2012;22:60–9. https://doi.org/10.1016/j.semcancer.2011.12.003.
13. Carmely A, Meirow D, Peretz A, Albeck M, Bartoov B, Sredni B. Protective effect of the immunomodulator AS101 against cyclophosphamide-induced testicular damage in mice. Hum Reprod. 2009;24:1322–9. https://doi.org/10.1093/humrep/den481.
14. Kalich-Philosoph L, Roness H, Carmely A, Fishel-Bartal M, Ligumsky H, Paglin S, Wolf I, Kanety H, Sredni B, Meirow D. Cyclophosphamide triggers follicle activation and "burnout"; AS101 prevents follicle loss and preserves fertility. Sci Transl Med. 2013;5:185ra62. https://doi.org/10.1126/scitranslmed.3005402.
15. Di Emidio G, Rossi G, Bonomo I, Alonso GL, Sferra R, Vetuschi A, Artini PG, Provenzani A, Falone S, Carta G, D'Alessandro AM, Amicarelli F, Tatone C. The natural carotenoid crocetin and the synthetic tellurium compound AS101 protect the ovary against cyclophosphamide by modulating SIRT1 and mitochondrial markers. Oxidative Med Cell Longev. 2017;2017:8928604. https://doi.org/10.1155/2017/8928604.
16. Pascuali N, Scotti L, Di Pietro M, Oubiña G, Bas D, May M, Gómez Muñoz A, Cuasnicú PS, Cohen DJ, Tesone M, Abramovich D, Parborell F. Ceramide-1-phosphate has protective properties against cyclophosphamide-induced ovarian damage in a mice model of premature ovarian failure. Hum Reprod. 2018;33:844–9. https://doi.org/10.1093/humrep/dey045.

17. Sonigo C, Beau I, Grynberg M, Binart N. AMH prevents primordial ovarian follicle loss and fertility alteration in cyclophosphamide-treated mice. FASEB J. 2019;33:1278–87. https://doi.org/10.1096/fj.201801089R.
18. Roness H, Spector I, Leichtmann-Bardoogo Y, Savino AM, Dereh-Haim S, Meirow D. Pharmacological administration of recombinant human AMH rescues ovarian reserve and preserves fertility in a mouse model of chemotherapy, without interfering with anti-tumoural effects. J Assist Reprod Genet. 2019;36:1793–803. https://doi.org/10.1007/s10815-019-01507-9.
19. Dittrich R, Lotz L, Fehm T, Krüssel J, von Wolff M, Toth B, van der Ven H, Schüring AN, Würfel W, Hoffmann I, Beckmann MW. Xenotransplantation of cryopreserved human ovarian tissue-a systematic review of MII oocyte maturation and discussion of it as a realistic option for restoring fertility after cancer treatment. Fertil Steril. 2015;103:1557–65. https://doi.org/10.1016/j.fertnstert.2015.03.001.
20. Snow M, Cox SL, Jenkin G, Trounson A, Shaw J. Generation of live young from xenografted mouse ovaries. Science. 2002;297:2227.
21. Lotz L, Liebenthron J, Nichols-Burns SM, Montag M, Hoffmann I, Beckmann MW, van der Ven H, Töpfer D, Dittrich R. Spontaneous antral follicle formation and metaphase II oocyte from a non-stimulated prepubertal ovarian tissue xenotransplant. Reprod Biol Endocrinol. 2014;12:41. https://doi.org/10.1186/1477-7827-12-41.
22. Fishman JA, Patience C. Xenotransplantation: infectious risk revisited. Am J Transplant. 2004;4:1383–90.
23. Telfer EE, Zelinski MB. Ovarian follicle culture: advances and challenges for human and nonhuman primates. Fertil Steril. 2013;99:1523–33. https://doi.org/10.1016/j.fertnstert.2013.03.043.
24. 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;24:135–42. https://doi.org/10.1093/molehr/gay002.
25. Vanacker J, Dolmans MM, Luyckx V, Donnez J, Amorim CA. First transplantation of isolated murine follicles in alginate. Regen Med. 2014;9:609–19. https://doi.org/10.2217/rme.14.33.
26. 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.
27. 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;35:41–8. https://doi.org/10.1007/s10815-017-1091-3.
28. Liverani L, Raffel N, Fattahi A, Preis A, Hoffmann I, Boccaccini AR, Beckmann MW, Dittrich R. Electrospun patterned porous scaffolds for the support of ovarian follicles growth: a feasibility study. Sci Rep. 2019;9:1150. https://doi.org/10.1038/s41598-018-37640-1.
29. 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;104:672–680.e2. https://doi.org/10.1016/j.fertnstert.2015.05.021.
30. Telfer EE, Anderson RA. The existence and potential of germline stem cells in the adult mammalian ovary. Climacteric. 2019;22:22–6. https://doi.org/10.1080/13697137.2018.1543264.
31. Brännström M, Enskog A, Kvarnström N, Ayoubi JM, Dahm-Kähler P. Global results of human uterus transplantation and strategies for pre-transplantation screening of donors. Fertil Steril. 2019;112:3–10. https://doi.org/10.1016/j.fertnstert.2019.05.030.
32. Brännström M, Dahm-Kähler P. Uterus transplantation and fertility preservation. Best Pract Res Clin Obstet Gynaecol. 2019;55:109–16. https://doi.org/10.1016/j.bpobgyn.2018.12.006.

Part IV
After Fertility Preservation

Treatment of Uterine Bleeding During Chemotherapy

Nicole Sänger, Michael von Wolff, and Frank Nawroth

Background

Abnormal bleeding as a result of myelosuppressive therapy is associated with significant morbidity in affected patients, which is why preventive concepts should be considered before gonadotoxic treatment is started. Clinically relevant are mainly menorrhagia/menometrorrhagia with a blood loss of >80 mL per cycle over >7 days, but also irregular, acyclic bleeding with considerable subsequent complications. In patients with thrombocytopenia, managing the bleeding disorder can be a challenging dilemma for the treating oncologists. In addition to deciding on the appropriate start of treatment (i.e. in acute cases or for prevention), drug options may also be limited due to contraindications.

Regularly used substances used alone or in combination:

- Oral contraceptives
- GnRH agonists
- Estrogens and progestogens including progestogens containing intrauterine devices (IUD)

N. Sänger (✉)
Department of Gynaecological Endocrinology and Reproductive Medicine, University Hospital Bonn, Bonn, Germany
e-mail: nicole.saenger@ukbonn.de

M. von Wolff
Department of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

F. Nawroth
Specialist Centre for Reproductive Medicine, Prenatal Medicine, Endocrinology and Osteology, amedes MVZ Hamburg, Hamburg, Germany
e-mail: Frank.Nawroth@amedes-group.com

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_28

- Tranexamic acid
- Blood products such as platelet concentrates, recombinant factor VIIa preparations etc.

There are no standard treatment recommendations as yet. Hormonal treatment concepts in the form of oral contraceptives are part of first-line therapy in everyday clinical practice, but the dosage (single or double dosage) and application regimen (cyclic vs. long cycle) can vary considerably. GnRH agonists (GnRHa) and tranexamic acid are also highly valued, whereas regular platelet concentrate use is rarely indicated due to the risk of alloimmunization. Surgical–interventional procedures are also discussed, especially in cases of treatment failure. These include curettage or balloon procedures, as used in obstetrics. Endometrial ablation or uterine artery embolization (UAE) should not be performed even in the acute situation, or only as a last resort, especially in premenopausal patients with a prospective desire to have children. In addition, UAE leads to the desired therapeutic result in the form of oligo- or amenorrhoea only after a delay.

The aim should be to adjust the patient's medication adequately according to her individual risk-benefit profile before the start of gonadotoxic therapy to prevent undesired events in addition to the already existing side effect profile of chemotherapy. This chapter highlights the most frequently administered medication and aims to show possible solutions to uterine bleeding disorder in myelosuppression.

Oral Contraceptives

Combined oral contraceptives (COC) can reduce menorrhagia/menometrorrhagia in premenopausal patients during myelosuppressive treatment and can lead to amenorrhoea after long-term use. They are often used clinically due to their good acceptance by both the treating and the affected person. They are also often used for contraception beforehand. However, there is a lack of controlled, randomized studies on the effectiveness and safety of chemotherapy-associated thrombocytopenic bleeding disorders in oncology patients [1]. The use of COCs is nevertheless associated with some safety concerns [2]. The use of COCs is associated with an additional increased risk of thromboembolic disease, especially in tumour patients and the associated hypercoagulability per se.

Classic progestogen-only-pills (POPs) (or the progestogen containing IUD), on the other hand, can lead to mutually unsatisfactory breakthrough and continuous bleeding if there is a understandable reason to avoid the estrogen component [3]. Nevertheless, a reduction in both the amount and duration of bleeding has been described for the use of pure progestogen preparations [4]

It should be noted that secondary symptoms of gonadotoxic treatment such as nausea, vomiting, diarrhoea and mucositis can lead to reduced absorption, thus inadequate dosage and insufficient activity of the COC.

Pharmacological evidence also shows that under certain circumstances, a dose adjustment may be necessary, especially for cytochrome P-metabolizing substances (including the COC), since they can be degraded more rapidly during the detoxification of the cytotoxic drugs.

Furthermore, the impairment of hepatic function and the existing risk of hepatotoxicity during chemotherapy with simultaneous use of COC should be considered, although it can be difficult to assess whether increased transaminases are related to cytotoxic therapy per se or the (re)-adjustment to the COC.

GnRH Agonists

The mode of action of GnRH agonists (GnRHa) has already been described in detail in chapter "GnRH Agonists". The induction of amenorrhoea using GnRHa to prevent bleeding disorders in thrombocytopenic phases of chemotherapy has been evaluated and documented as a possible treatment option in several studies [3–9].

In contrast, few clinical studies exist comparing GnRHa with COC in oncological patients [5]. While COC can lead to a rapid reduction in menorrhagia and the cycle can quickly regulate itself again after discontinuation of medication, longer administration of GnRHa is often required before gonadotoxic therapy before the desired amenorrhoea occurs. However, due to its mode of action, the effect lasts for a long period of time even after the end of chemotherapy. There does not appear to be a significant difference in the degree of liver toxicity, at least not according to current data, in patients undergoing simultaneous chemotherapy and in oncological patients.

It is now assumed that GnRHa can also be started shortly before chemotherapy.

With GnRHa therapy alone, a later estrogen withdrawal bleeding, around 1 week after the first GnRHa injection, must be expected due to the initial flare-up effect, which could potentially fall into the nadir. Studies have shown that the simultaneous, continuous administration of a progestogen within the first month of GnRHa administration can prevent this problem [6, 7]. Ideally, both the GnRHa and the progestogen are administered 1 month before the start of chemotherapy, although this can only be realistically implemented in the case of a planned stem cell transplant.

The side effect profile of GnRHa should be considered and discussed with the patient, especially if estrogen substitution is not planned. These include hot flushes, nausea, vomiting, allergic reactions, infections and necrosis at the injection site as well as a reduction in bone density. Due to the reduction of bone density, it is recommended that GnRHa administration should not be continued for more than 6 months if possible. If symptoms are severe, GnRHa can be combined (in the case of hormonally inactive tumours) with an estrogen therapy.

Estrogens and Progestogens

If bleeding lasts for more than 2 weeks, the endometrium is usually atrophic and sonographically thin. In this situation, stabilization of the endometrium is best achieved with pure high-dose oestrogen therapy. Since high-dose oral therapy can also lead to nausea and, as described for oral contraceptives, absorption can be reduced and the liver can be additionally burdened by the first -pass metabolism, transcutaneous administration should be considered during chemotherapy.

It should be noted that an additional progestogen must be administered 1–2 weeks after the start of estrogens to enable transformation of the built-up endometrium and a regulated menstrual bleed.

If the endometrium is thick, bleeding may result from insufficient transformation, and pure progestogen administration is recommended.

If ultrasound measurement of endometrial thickness is not possible, simultaneous estrogen/progestogen therapy should be considered. This can be done with the preparations described in Table 1, but also with oral, single-phase contraceptives,

Table 1 Examples of drug treatments for acute bleeding disorders during treatment with gonadotoxic agents

Active substance	Dosage	Duration of use	Onset of action	Comments
Oral estrogens	6–8 mg micronized estradiol (valerate)/day until bleeding is only minimal (ca. 24–48 h), then 1–2 × 2mg estradiol (valerate) /day	Maximum 3 weeks, afterwards additional progestin, e.g., MPA: 1 × 10 mg oral/ day for 10 days	Bleeding stops around 10 h after start of treatment	To be used especially if endometrium is thin. Cave: Risk of thrombosis increased
Trans-cutaneous estrogens	200 µg transdermal estradiol /day	Same as for oral estrogens	Bleeding stops around 10 h after start of treatment	To be used especially if endometrium is thin. Risk of thrombosis lower compared to oral estrogens
Oral progestins	– Medroxyprogesterone acetate (MPA): 2 × 10–20 mg /day – Chlormadinone acetate (CMA): 2-4 mg /day or – Norethisterone acetate (NETA): 1-2 × 5mg/d	Minimum 10 days	Bleeding stops around 72 h after start of treatment	To be used especially if endometrium is thick
Oral anti-fibrinolytics	Tranexamic acid: 3–4 × 1–1.5g/d	One to few days	Bleeding stops after 2–3 h	Cave: elevated risk of thrombosis possible

e.g. those containing levonorgestrel (see above). However, these must be started at an initially higher dosage (e.g. day 1: 3 tablets, day 2: 2 tablets, from day 3: 1 tablet), which can lead to nausea and an increased risk of thrombosis.

Intrauterine progestogen containing IUDs are used to prevent bleeding during chemotherapy. For this reason, they do not have to be removed before chemotherapy.

Tranexamic Acid

The best data on the reduction of excessive uterine bleeding are available from randomized studies with tranexamic acid, although the inclusion criteria did not apply to oncology patients [10]. Side effects have rarely been described and were mostly dysmenorrhoea, headache and back pain. The relationship between tranexamic acid substitution and the risk of venous thrombosis or pulmonary embolism (VTE) was also investigated [11]. However, the evaluation, considering all VTE-increasing factors such as simultaneous use of oral contraceptives, surgical intervention, chronic inflammatory bowel disease, systemic lupus erythematosus (SLE), obesity and smoking, did not reveal any significant increase in risk. It should be noted that, as already mentioned, these studies were not performed on oncology patients with menorrhagia/menometrorrhagia and consequent hypercoagulability, and this will probably not be carried out in the future.

A dosage of at least 3–4 × 1–1.5 g/day orally (Table 1) led to a significant reduction in the amount of bleeding [10, 12].

Other Treatment Agents

Substances such as danazol and desmopressin are not that common. Ulipristal acetate is also hardly ever prescribed to oncology patients, despite rapid cessation of uterine bleeding in more than 90% and within 5–7 days [13, 14].

Ulipristal acetate belongs to the group of selective progesterone receptor modulators with both agonistic and antagonistic properties. It fixes mid-cycle estrogen levels without the known side effect spectrum of GnRHa and showed comparable effects on bleeding in patients with uterine fibroids compared to GnRHa application [15]. Due to the potential hepatotoxicity, a careful evaluation in the described patient population is required.

Practical Approach

Prevention of Vaginal Bleeding (Fig. 1)

- If there is enough time before the start of chemotherapy: oral combined single-phase contraceptives with an estrogen component of about 30 µg in combination with a progestogen with a low risk of thrombosis and a partial anti-oestrogenic effect, e.g. levonorgestrel.
- If there is a high risk of thrombosis: Progestogen monotherapy using a progestogen with a low risk of thrombosis and a partial anti-oestrogenic effect, e.g. medroxyprogesterone acetate (MPA): 1-2 × 10 mg/day.
- Also possible and effective, but more expensive: GnRHa, especially under the simultaneous aspect of fertility protection (see chapter "GnRH Agonists"). Start of treatment 1 week before the start of chemotherapy. According to experts even a shorter interval is possible. Additional estrogens may be necessary for hypoestrogenic symptoms, e.g. 25–50 µg estradiol transdermally or 1–2 mg estradiol(valerate)/day.

Treatment of Acute Vaginal Bleeding During Chemotherapy (Table 1, Fig. 1)

- Oral tranexamic acid: tranexamic acid acts quickly but has a short half-life, so it must be administered repeatedly. Although an increased risk of thrombosis has not been confirmed in clinical studies, it cannot be ruled out in oncology patients (who have not yet been investigated). Dosage: 3–4 × 1–1.5 g/day.
- Oral or transcutaneous estrogens and/or progestogens depending on the thickness of the endometrium measured using ultrasound. For dosages see Table 1.
- Oral progestogen monotherapy if there is a high risk of thrombosis. For dosage see Table 1.

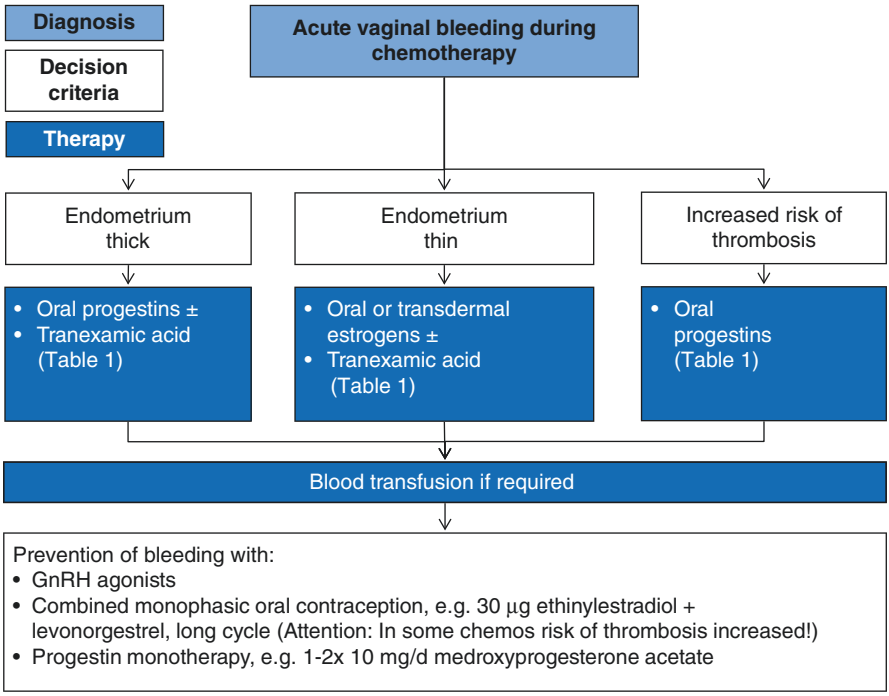


Fig. 1 Treatment decision flowchart for acute vaginal bleeding during chemotherapy

References

1. Bates JS, Buie LW, Woodis CB. Management of menorrhagia associated with chemotherapy-induced thrombocytopenia in women with hematologic malignancy. Pharmacotherapy. 2011;31:1092–110.
2. Purisch SE, Shanis D, Zerbe C, Merideth M, Cuellar-Rodriguez J, Stratton P. Management of uterine bleeding during hematopoietic stem cell transplantation. Obstet Gynecol. 2013;121(2 Pt 2 Suppl 1):424–7.
3. Chiusolo P, Salutari P, Sica S, Scirpa P, Laurenti L, Piccirillo N, et al. Luteinizing hormone-releasing hormone analogue: leuprorelin acetate for the prevention of menstrual bleeding in premenopausal women undergoing stem cell transplantation. Bone Marrow Transplant. 1998;21:821–3.
4. Boonyawat K, O'Brien SH, Bates SM. How I treat heavy menstrual bleeding associated with anticoagulants. Blood. 2017;130:2603–9.
5. Sica S, Salutari P, Di Mario A, Chiusolo P, Rutella S, La Barbera EO, et al. Treatment and prophylaxis of hypermenorrhea with leuprorelin in premenopausal women affected by acute leukemia at diagnosis. Am J Hematol. 1996;51:248–9.
6. Lhommé C, Brault P, Bourhis JH, Pautier P, Dohollou N, Dietrich PY, et al. Prevention of menstruation with leuprorelin (GnRH agonist) in women undergoing myelosuppressive chemotherapy or radiochemotherapy for hematological malignancies: a pilot study. Leuk Lymphoma. 2001;42:1033–41.

7. Laufer MR, Townsend NL, Parsons KE, Brody KA, Diller LR, Emans SJ, et al. Inducing amenorrhea during bone marrow transplantation. A pilot study of leuprolide acetate. J Reprod Med. 1997;42:537–41.
8. Meirow D, Rabinovici J, Katz D, Or R, Shufaro Y, Ben-Yehuda D. Prevention of severe menorrhagia in oncology patients with treatment-induced thrombocytopenia by luteinizing hormone-releasing hormone agonist and depo-medroxyprogesterone acetate. Cancer. 2006;107:1634–41.
9. Ghalie R, Porter C, Radwanska E, Fitzsimmons W, Richman C, Kaizer H. Prevention of hypermenorrhea with leuprolide in premenopausal women undergoing bone marrow transplantation. Am J Hematol. 1993;42:350–3.
10. Lukes AS, Moore KA, Muse KN, Gersten JK, Hecht BR, Edlund M, et al. Tranexamic acid treatment for heavy menstrual bleeding: a randomized controlled trial. Obstet Gynecol. 2010;116:865–75.
11. Sundström A, Seaman H, Kieler H, Alfredsson L. The risk of venous thromboembolism associated with the use of tranexamic acid and other drugs used to treat menorrhagia: a case-control study using the general practice research database. BJOG. 2009;116:91–7.
12. Lukes AS, Muse K, Richter HE, Moore KA, Patrick DL. Estimating a meaningful reduction in menstrual blood loss for women with heavy menstrual bleeding. Curr Med Res Opin. 2010;26:2673–8.
13. Donnez J, Tatarchuk TF, Bouchard P, Puscasiu L, Zakharenko NF, Ivanova T, et al. Ulipristal acetate versus placebo for fibroid treatment before surgery. N Engl J Med. 2012;366:409–20.
14. Donnez J, Tomaszewski J, Vázquez F, Bouchard P, Lemieszczuk B, Baró F, et al. Ulipristal acetate versus leuprolide for fibroid treatment before sugery. N Engl J Med. 2012;366:421–32.
15. Estradella J, Español P, Ascencio F, Perelló J, Calaf J. Ulipristal acetate for the management of acute heavy menstrual bleeding without fibroids. Gynecol Endocrinol. 2018;34:554–7.

Fertility Treatment After Fertility Preservation Therapies

Michael von Wolff

Introduction

Spontaneous conception should be tried if possible after a fertility preservation measure, since assisted reproductive techniques are associated with greater effort, more treatment-induced stress and, when in vitro fertilization (IVF) is carried out, with an increased risk of childhood malformations [1]. The fact that there is no evidence of an increased malformation rate in children after gonadotoxic treatment also speaks in favour of spontaneous conception [2]. However, from a reproductive-biological point of view, the desire to have children should be realized 6 months at the earliest after completion of chemotherapy, as the chemotherapy washout phase and a cycle of spermatogenesis and oogenesis should be awaited. From an oncological point of view, it is usually necessary to wait much longer than 6 months. Fertility therapy is only possible if pregnancy is compatible with the underlying disease and if the oncologist has approved a pregnancy.

If spontaneous conception is tried when the ovarian reserve is low, a fertility assessment should be carried out at an early stage (semen analysis, examination of tubal patency, exclusion of endocrinological abnormalities, etc.) so that pathologies can be taken into account during fertility treatment. If the menstrual cycle is irregular, an attempt can be made to monitor the cycle using ultrasound and/or hormonal examinations to prove folliculogenesis and to optimize the timing of sexual intercourse. Intrauterine insemination should be considered for low-grade andrological subfertility, and IVF or ICSI should be indicated if there is highly impaired sperm quality. If POI or azoospermia is present or pregnancy does not occur spontaneously, the cryopreserved germ cells or gonadal tissue can be used.

M. von Wolff (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_29

Table 1 Utilization rates of oocytes, ovarian tissue and sperm after cryopreservation as a fertility preservation measure

	Patients with cryopreservation, *n*	Patients with at least one embryo transfer, *n* (%)	Patients with at least one tissue transplantation, *n* (%)	Patients who used cryopreserved sperm, *n* (%)
Oocytes [3, 4]	2097	129/2097 (6.1%)		
Ovarian tissue [4–6]	3845		114/3845 (2.9%)	
Sperm [7]	11,798			974/11798 (8.3%)

Utilization of Cryopreserved Gametes and Tissue

Cryopreserved gametes and gonadal tissue are only thawed up if infertility occurs as a result of oncological or non-oncological treatment. Accordingly, the utilization rate of cryopreserved preparations is relatively low (Table 1). However, it can be assumed that this will increase for oocytes and ovarian tissue, as many years often pass before the desire to have children. Nevertheless, the data show that the indication for cryopreservation should not be made too generously (see chapter "Indications for and Against Fertility Preservation") to avoid too many unnecessary cryopreservation procedures.

Success Rates

Success rates after using cryopreserved oocytes (see chapter "Cryopreservation of Unfertilized and Fertilized Oocytes"), ovarian tissue (see chapter "Transplantation of ovarian tissue") and sperm (see chapter "Cryopreservation of Sperm and Testicular Tissue") are strongly dependent on the number and quality of the gametes or ovarian tissue. Table 2 provides an indicative prognosis of the success rates. According to this, about one-third of the women have a child after using cryopreserved oocytes or ovarian tissue. With cryopreserved sperm, this is approximately one-half of the couples.

However, the success rate per cryopreservation performed is very low (Table 2) and is below 5% for all techniques due to the low retrieval rate. Detailed data on success rates are presented for oocytes in chapter "Cryopreservation of Unfertilized and Fertilized oocytes", for ovarian tissue in chapter "Transplantation of ovarian tissue" and for sperm in this chapter in Table 3.

Table 2 Success rates after using frozen oocytes, ovarian tissue and sperm after cryopreservation as a fertility preservation measure

	Success rates	Patients with at least one birth per total number of cryopreservations performed, *n* (%)
Oocytes [3, 4]	*Pregnancy rate per transfer:* (1.4 embryos): 38.9%. *Birth rate per transfer:* (1.4 embryos): 30.0%. *Patients with at least one birth after thawing oocytes:* 35.2% [3], 32.6% [4]	41/2097 (2.0%)
Ovarian tissue [4–6]	*Patients with at least one pregnancy after transplantation:* 32.7% [4], 27.3% [3], 33.3% [5]. *Patients with at least one birth after transplantation:* 30.3% [5], 18.2% [4], 33.3% [6].	30/3845 (0.8%)
Sperm [7]	*Pregnancy rate per therapy cycle:* – per intrauterine insemination (IUI): 13% – per IVF cycle: 30% *Birth rate per therapy cycle:* – per IUI: 8% – per IVF cycle: 25% *Couples with at least one birth after sperm use:* 49%	237/5461 (4.4%)

Table 3 Clinical pregnancy rates after intrauterine insemination (IUI) depending on the total number of inseminated, non-cryopreserved sperm

	Cycles studies, *n*	Pregnancy rates depending on the number of inseminated, motile sperm ($\times 10^6$)							
		<1	<2	1–<2	1–4	2–<5	5–9	5–<10	≥10
Wainer et al. [8]	2564	3.1%		8.7%		11.9%		14.8%	13.1%
Cao et al. [9]	1153		4.1%			15.6%		12.7%	15.0%
Gubert et al. [10]	2062	3.8%			12.7%		12.2%		16.7%

Use of Cryopreserved Sperm

Cryopreserved sperm (see chapter "Cryopreservation of Sperm and Testicular Tissue") can be accessed if treatment-induced andrological sterility is present. If a large number of motile sperm have been cryopreserved, several attempts at intrauterine insemination (IUI) can be made. However, in the event of male azoospermia, the sperm deposit should not be completely used so that IVF can be carried out at a later date if pregnancy does not occur after IUI. If the quality of the sperm depot is limited, IVF or intracytoplasmic sperm injection (ICSI) is the primary option.

Table 3 shows the success rates of IUI in infertile couples without prior fertility preservation therapy and without cryopreservation. The data show that at least 1 million motile sperm should be present for an insemination attempt.

Use of Cryopreserved Oocytes

After cryopreservation, oocytes must first be thawed and fertilized (see chapter "Cryopreservation of Unfertilized and Fertilized Oocytes"). Fertilization is performed by ICSI. The transfer can take place both in the spontaneous cycle or after building up the endometrium with estrogen/progesterone. Although both techniques are equivalent in terms of the success rate in patients with eumenorrhoea [11], endometrial build-up with estrogens/progesterone is often preferred because it is easier to plan.

In principle, the therapeutic approach is not different to IVF in infertile couples. Data on the success rate can be found in Table 2 and chapter "Cryopreservation of Unfertilized and Fertilized Oocytes", Table 2.

Use of Cryopreserved Ovarian Tissue

After cryopreservation (see chapter "Transport, Cryopreservation and Storage of Ovarian Tissue") and transplantation of ovarian tissue (see chapter "Transplantation of Ovarian Tissue"), it takes about 2–3 months for the primordial and primary follicles to grow into mature follicles. Due to the high FSH concentration in the blood, a larger cohort of follicles can be generated to begin with, which initially leads to multifollicular growth. Ideally, this first cohort is used for fertility therapy, either by hCG administration and timed sexual intercourse or by follicle aspiration. The risk of a multiple pregnancy seems to be very low.

The fundamental question is whether the aim should be a spontaneous or an IVF pregnancy. According to studies published to date, spontaneous pregnancies are more common than IVF pregnancies (Table 4). Even centres that prefer IVF report

Table 4 Spontaneous and IVF pregnancies after transplantation of ovarian tissue

	Transplanted patients, *n*	Spontaneous pregnancies, *n*	IVF pregnancies, *n*
Van der Veen et al. [5]	49	18	3
Meirow et al. [12]	20	6	13
Diaz-Garcia et al. [4]	44	5	5
Fortin et al. [13]	34	9	1
Total	**147**	**38**	**22**

spontaneous conception between IVF cycles. It is therefore advisable to ensure at the time of tissue transplantation that no additional sterility factors (pathological sperm, blocked fallopian tubes, endocrine pathologies, etc.) are present.

It should be noted that menstrual cycles after tissue transplantation cannot be compared with normal spontaneous cycles. The ovarian reserve is very low due to the small amount of transplanted tissue and tissue loss in the first weeks after transplantation. Because of this:

- Menstrual cycles are often irregular
- There is usually at least a temporary increase of gonadotropin concentration in serum
- Follicle maturation disorders are possible
- Premature ovulation may occur

M. Nitzschke (from Cryocan) developed a model of different stages of POI, which are shown in Table 5 from which treatment recommendations can be derived.

- If the ovarian reserve is not too low because a larger amount of tissue has been transplanted, which can be demonstrated by a slightly increased AMH concentration, then gonadotrophin stimulation can be performed.
- If, however, the ovarian reserve is very low and there is imminent POI with a compensation stage, then natural cycle IVF, as described in detail elsewhere ([14], www.IVF-Naturelle.com), is a suitable option.
- If there is follicular–endometrial desynchronization caused by early follicular growth in the previous luteal phase (due to tendentially elevated basal FSH concentrations), an attempt can be made to suppress luteal FSH release by combined, single-phase estrogen/progestogen-containing contraceptives or progestogens.
- If a premature increase in LH with still immature follicles occurs, early administration of GnRH antagonists (GnRHant), e.g. starting from a follicle size of 13–14 mm, can be used. If GnRHant is administered for longer than 1 day, the associated reduction in FSH release must be compensated for by daily injection of e.g. 50–75 IU gonadotrophins.

As a result of the cycle changes mentioned above, the ideal time for follicle aspiration is difficult to predict. Aspiration is therefore often performed very early when the follicles are still small. Gook et al. [15] aspirated at an average follicle size of 14 mm. The follicles were not flushed due to their small size. However, flushing result in a higher egg yield and transfer probability with monofollicular growth (Table 6, [16]). Compared to women without an ovarian tissue transplant, both the number of metaphase II oocytes obtained and the fertilization rate are lower in women in which follicles from transplanted tissue were aspirated. Although the oocyte yield could be increased by flushing the follicles (Table 6, [16]), the effectiveness of IVF is likely to remain low.

Because of increased gonadotropin concentrations the associated risk of premature ovulation or premature luteinization of the follicles, some centres prefer downregulation with GnRH antagonists from a follicle size of 13 mm at the latest in

Table 5 Model of a stage classification of premature ovarian insufficiency (POI) and derived therapeutic intervention options (Reproduced with permission from M. Nitzschke, Cryocan and M. von Wolff)

Stage	Pathophysiology	Possible clinical characteristics	Frequent hormone concentrations	Possible therapeutic intervention
Compensation	Ovarian reserve low; Cycle length normal	Cycle length normal; Follicular phase normal; Luteal phase normal	AMH very low/ undetectable; Basal estradiol normal; Basal FSH concentration normal	Natural cycle IVF without gonadotrophin stimulation; IVF with gonadotrophin stimulation if more than 1 follicle is expected
Desynchronization	Premature follicle growth already during the late luteal phase/ menstruation; Endometrial proliferation phase shortened	Cycle length shortened; Follicular phase shortened; Luteal phase normal	AMH very low/ undetectable; Basal estradiol increased; Basal FSH concentration normal/slightly increased	Natural cycle IVF, combined pre-cycle estrogen/progestin contraceptives to suppress premature luteal folliculogenesis
Premature ovulation	Premature LH rise	Cycle length shortened; Follicular phase shortened; Luteal phase insufficient	AMH very low/ undetectable; Basal estradiol normal; Basal FSH concentration increased; Premature LH surge	Natural cycle IVF; early administration of GnRH antagonists ± gonadotrophins
Decompensation	No or only irregular follicle growth	Cycle length extended/ Amenorrhoea; Follicular phase extended; Luteal phase normal or insufficient	AMH undetectable; Estradiol low, Basal FSH concentration very high	IVF not possible

combination with gonadotrophin stimulation (e.g. 75 IU every second day [14] or 225 IU human menopausal gonadotropin (hMG) daily [12]). However, the associated therapeutic effort and costs are considerable.

Because of these difficulties, attempting spontaneous conception is a practicable approach. To improve the chances of success, cycle monitoring combined with hCG administration to trigger ovulation, followed by timed sexual intercourse, may be considered.

IVF should only be performed primarily if pregnancy does not occur within 1/2 to 1 year after transplantation or if other sterility factors are also present.

Table 6 Comparison of the effectiveness of IVF after transplantation of ovarian tissue with natural cycle IVF in infertile women without transplantation and without previous malignant diseases

	IVF in women after orthotopic transplantation of ovarian tissue (*without* follicle flushing) [15]	Natural cycle IVF in fertility patients (*without* follicle flushing) [16]	*P*-value	Natural cycle IVF in fertility patients (*with* follicle flushing) [16]
Mean age of women (years)	28.4 (time of cryopreservation)	35.0		35.0
Aspirated follicles, *n*	**108**	**81**		**83**
Mean follicle size, mm	14.3	18		18
Total oocytes, n	68 (63.0%)	51 (63.0%)		69 (83.1%)
Metaphase II-oocytes, *n*	47 (43.5%)	48 (59.3%)	< 0.05	64 (77.1%)
Oocytes with fertilization attempt, *n*	**53**	**48**		**64**
Fertilized oocytes (day 2), *n*	28 (52.8%)	38 (79.2%)	< 0.05	52 (81.8%)
Transferred embryos, *n*	**20**	**38**		**52**
Pregnancies per transfer, *n*	5 (25.0%)	10 (26.3%)	n.s.	12 (23.1%)

References

1. von Wolff M, Haaf T. In vitro fertilization technology and child health—risks, mechanisms and possible consequences. Dtsch Arztebl Int. 2020;117:23–30.
2. Meistrich ML, Byrne J. Genetic disease in offspring of long-term survivors of childhood and adolescent cancer treated with potentially mutagenic therapies. Am J Hum Genet. 2002;70:1069–71.
3. Cobo A, García-Velasco JA, Domingo J, Pellicer A, Remohí J. Elective and onco-fertility preservation: factors related to IVF outcomes. Hum Reprod. 2018;33:2222–31.
4. Diaz-Garcia C, Domingo J, Garcia-Velasco JA, Herraiz S, Mirabet V, Iniesta I, et al. Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for adult women undergoing gonadotoxic treatments: a prospective cohort study. Fertil Steril. 2018;109:478–85.e2.
5. Van der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krüssel J, et al. FertiPROTEKT network. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. Hum Reprod. 2016;31:2031–41.
6. Jadoul P, Guilmain A, Squifflet J, Luyckx M, Votino R, Wyns C, et al. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. Hum Reprod. 2017;32:1046–54.
7. Ferrari S, Paffoni A, Filippi F, Busnelli A, Vegetti W, Somigliana E. Sperm cryopreservation and reproductive outcome in male cancer patients: a systematic review. Reprod Biomed Online. 2016;33:29–38.

8. Wainer R, Albert M, Dorion A, Bailly M, Bergère M, Lombroso R, et al. Influence of the number of motile spermatozoa inseminated and of their morphology on the success of intrauterine insemination. Hum Reprod. 2004;19:2060–5.
9. Cao S, Zhao C, Zhang J, Wu X, Zhou L, Guo X, et al. A minimum number of motile spermatozoa are required for successful fertilisation through artificial intrauterine insemination with husband's spermatozoa. Andrologia. 2014;46:529–34.
10. Gubert PG, Pudwell J, Van Vugt D, Reid RL, Velez MP. Number of motile spermatozoa inseminated and pregnancy outcomes in intrauterine insemination. Fertil Res Pract. 2019;5:10.
11. Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev. 2017;7:CD003414.
12. Meirow D, Ra'anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertil Steril. 2016;106:467–74.
13. Fortin A, Azaïs H, Uzan C, Lefebvre G, Canlorbe G, Poirot C. Laparoscopic ovarian tissue harvesting and orthotopic ovarian cortex grafting for fertility preservation: less is more. Fertil Steril. 2019;111:408–10.
14. von Wolff M. The role of natural cycle IVF in assisted reproduction. Best Pract Res Clin Endocrinol Metab. 2019;33:35–45.
15. Gook D, Hale L, Polyokov A, Stern K. Outcomes from heterotopic and orthotopic grafting of human cryopreserved ovarian tissue. Hum Reprod. 2019;34(Suppl):i60–1.
16. Kohl Schwartz, A, Calcaferri I, Roumet M, Fink A, Wuuest A, Weidlinger S et al. Follicular flushing leads to higher oocyte yield in monofollicular natural cycle IVF – a prospective randomized controlled trial. Hum Reprod, in press.

Pregnancy After Chemotherapy and Pelvic Radiotherapy

Michael von Wolff

Effects of Chemotherapy

The effects of chemotherapy on a later pregnancy seem to be generally rather small. An increased risk of malformations has not been described after previous chemotherapy [1]. However, the risk of early miscarriage seems to be somewhat higher [2, 3]. According to a study from the 1980s, other pregnancy pathologies such as premature birth and low birth weight only occur more frequently if the pregnancy occurred within the first year after completion of chemotherapy [4].

However, the data are not clear when chemotherapy is administered during childhood. In some studies, no adverse effect was found. However, Van de Loo et al. [5] described an increased risk of preterm birth (<37 weeks gestation) after oncological therapy in childhood without radiotherapy (and thus predominantly after chemotherapy) (OR 4.21, 95% CI, 1.02–17.32). Van de Loo et al. [5] attempted to resolve the controversial data situation to the effect that chemotherapy with alkylating agents such as busulfan [6] seems to lead to a reduced uterine volume and thus to an increased risk of pregnancy pathologies.

Effects of Radiotherapy

The effect of pelvic radiotherapy depends strongly on the radiation intensity and the age at radiation exposure, so that it is hardly possible to describe general effects.

M. von Wolff (✉)
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_30

Van de Loo et al. [5] investigated the effect of abdominal/pelvic irradiation during childhood (n = 55) on uterine volume and subsequent pregnancies. Data on radiation dose were not available in this study. Fifty-one percent of the women who received radiotherapy in childhood had a small uterus (<44.3 mL). In a non-oncological comparison group, the proportion was only 19%. After oncological therapy (regardless of whether with or without radiotherapy) the risks were increased compared to a non-oncological control group for both preterm birth (<7 weeks) (OR 10.31, 95% CI 1.68–63.18) and low birth weight (<2500 g) (OR 19.86, 95% CI 1.90–207.58). The risks tended to be even higher after radiotherapy.

Data on pregnancy risks in relation to a specific radiation dose were examined by Salooja et al. [7]. They compared women with a stem cell transplantation with and without total body irradiation. They analysed 28 pregnancies in 21 women (9 women after assisted reproduction) after total body irradiation (median 10 Gy, 7–12 Gy) compared to 58 pregnancies in 46 women without radiotherapy. Among the women with total body irradiation, the proportion of children born with a birth weight < 2500 g was about 30% compared to 10% without irradiation.

Data on pregnancies following direct pelvic radiotherapy are only available sporadically. Hürmüz et al. [8] reported a spontaneous singleton pregnancy at the age of 26 years with a Caesarean section in the 39th week of pregnancy after radiotherapy of the pelvis with 30 Gy to treat anal carcinoma at the age of 25 years.

De Menezes et al. [9] described a twin pregnancy after egg donation at the age of 31 years after radiotherapy to the right pelvis with 36 Gy to treat Hodgkin's lymphoma at 16 years. A Caesarean section was performed in the 35th week of pregnancy due to pre-eclampsia. Right-sided adherence of the placenta was found during surgery, which was attributed to the radiotherapy.

Bath et al. [10] reported a complication-free spontaneous singleton pregnancy at the age of 20 years with a Caesarean section in the 38th week of gestation to deliver a baby weighing 2950 g after radiotherapy to the left pelvis with 55 Gy and the right pelvis with 10 Gy to treat Ewing's sarcoma at 16 years of age.

Teh et al. [11] systematically analysed the clinical consequences of radiotherapy in childhood and adulthood and came to the following conclusions:

- Radiation during childhood seems to have a greater negative effect on the uterus than in adulthood.
- Radiotherapy to the adult uterus with whole-body radiation (12 Gy) is associated with an increased risk of abortion, premature birth and a low birth weight.
- If the uterus is irradiated with >25 Gy in childhood, pregnancy should not be recommended.
- If the uterus is irradiated with >45 Gy in adults, pregnancy should not be advised.

References

1. Meistrich ML, Byrne J. Genetic disease in offspring of long-term survivors of childhood and adolescent cancer treated with potentially mutagenic therapies. Am J Hum Genet. 2002;70:1069–71.
2. Velentgas P, Daling JR, Malone KE, Weiss NS, Williams MA, Self SG, et al. Pregnancy after breast carcinoma: outcomes and influence on mortality. Cancer. 1999;85:2424–32.
3. Lawrenz B, Henes M, Neunhoeffer E, Fehm T, Huebner S, Kanz L, et al. Pregnancy after successful cancer treatment: what needs to be considered? Onkologie. 2012;35:128–32.
4. Mulvihill JJ, McKeen EA, Rosner F, Zarrabi MH. Cancer. Pregnancy outcome in cancer patients. Experience in a large cooperative group. Cancer. 1987;60:1143–50.
5. van de Loo LEXM, van den Berg MH, Overbeek A, van Dijk M, Damen L, Lambalk CB, et al. Uterine function, pregnancy complications, and pregnancy outcomes among female childhood cancer survivors. Fertil Steril. 2019;111:372–80.
6. Beneventi F, Locatelli E, Giorgiani G, Zecca M, Mina T, Simonetta M, Cavagnoli C, et al. Adolescent and adult uterine volume and uterine artery Doppler blood flow among subjects treated with bone marrow transplantation or chemotherapy in pediatric age: a case-control study. Fertil Steril. 2015;103:455–61.
7. Salooja N, Szydlo RM, Socie G, Rio B, Chatterjee R, Ljungman P, et al. Late effects working party of the European Group for blood and marrow transplantation. Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey. Lancet. 2001;358:271–6.
8. Hürmüz P, Sebag-Montefiore D, Byrne P, Cooper R. Successful spontaneous pregnancy after pelvic chemoradiotherapy for anal cancer. Clin Oncol (R Coll Radiol). 2012;24:455–7.
9. De Menezes E, Tuck SM. Pelvic radiotherapy damage to the endometrium causing morbid adherence of placenta. A new risk factor? J Obstet Gynaecol. 2007;27:526–7.
10. Bath LE, Tydeman G, Critchley HO, Anderson RA, Baird DT, Wallace WH. Spontaneous conception in a young woman who had ovarian cortical tissue cryopreserved before chemotherapy and radiotherapy for a Ewing's sarcoma of the pelvis: case report. Hum Reprod. 2004;19:2569–72.
11. Teh WT, Stern C, Chander S, Hickey M. The impact of uterine radiation on subsequent fertility and pregnancy outcomes. Biomed Res Int. 2014;2014:482968.

Premature Ovarian Insufficiency: Hormone Replacement Therapy and Follow-Up

Volker Ziller, Petra Stute, and Michael von Wolff

Definitions, Causes, Signs and Symptoms, Diagnosis and Fertility

Definitions of POI and Early Menopause

- Premature ovarian insufficiency (POI): oligo-/amenorrhoea for at least 4 months and serum FSH > 25 IU/I measured twice at an interval of at least 4 weeks in women before the age of 40 [1], frequency: approximately 1% in women <40 years and 0.5% <35 years.
- Early menopause: menopause between 40th and 45th year of life, frequency: approx. 5% [1].

The frequency of POI or early menopause after gonadotoxic treatment depends in particular on the patient's age, type of chemotherapy as well as the radiotherapy dose (see chapter "Indications for and Against Fertility Preservation" and chapters of Part II).

V. Ziller (✉)
Dep. Gyn. Endocrinology and Reproductive Medicine, Clinic for Gynaecology and Obstetrics, University Hospital Giessen and Marburg (UKGM), Marburg, Germany
e-mail: ziller@med.uni-marburg.de

P. Stute · M. von Wolff
Division of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Bern, Bern, Switzerland
e-mail: Petra.Stute@insel.ch; Michael.vonWolff@insel.ch

M. von Wolff, F. Nawroth (eds.), *Fertility Preservation in Oncological and Non-Oncological Diseases*, https://doi.org/10.1007/978-3-030-47568-0_31

Causes of POI

In addition to the effects of chemo- or radiotherapy, chromosomal or genetic defects, autoimmunological processes, infection or even surgical interventions are discussed as causes of premature depletion of ovarian function. However, a large proportion of cases ultimately remains unexplained and must be classified as idiopathic [2–4].

The ovary is the most sensitive part of the female reproductive organs to chemotherapy. While the uterine function, for example, remains largely unaffected, alkylating substances in particular cause irreversible damage to the ovary and the different effects are intertwined. Genetic damage to the oocytes and the somatic cells of the resting and growing primordial follicles leads to pronounced follicle loss. In addition to these direct toxic effects, indirect effects are also suggested, e.g. by damage to the vascularization. The reduction in microvascularization leads to fibrosis and obliteration of both medullary and cortical structures [5].

Another possible mechanism is described in the "burnout" hypothesis. Cyclophosphamide, for example, appears to activate signalling pathways that could lead to a premature activation of the primordial follicles and thus to increased "consumption" [5].

Clinical Signs and Symptoms of POI

Women with POI may show typical symptoms of the menopause. Cycle disorders (initially polymenorrhoea, later oligo−/amenorrhoea) are followed by hot flushes, vaginal dryness, sleep disorders and psychological changes such as nervousness, irritability, concentration disorders and fatigue. In addition, there may be reduced libido, muscle and joint complaints and an increase in urogynaecological complaints.

The symptoms are very variable in their manifestation and can occur temporarily or even only partially. Young women with primary POI often exhibit significantly less symptoms compared to women with surgical menopause or sudden drug-induced estrogen withdrawal. This illustrates that the short-term symptoms are primarily caused by estrogen deprivation rather than relative estrogen deficiency [6].

In addition, however, the long-term consequences of estrogen deficiency must be considered. Besides sterility, these are mainly effects on bone metabolism, the cardiovascular system and changes in the nervous system [6].

Diagnosis of POI

Women with menstrual disorders should have a more detailed history taken of menopausal symptoms/complaints, as they often do not report these themselves (e.g. with the Menopause Rating Scale II (MRS-II)). The diagnosis of premature

ovarian insufficiency is then based on the combination of oligo-/amenorrhoea for at least 4 months and a serum FSH > 25 IU/I measured twice at an interval of at least 4 weeks, in women before the 40 years [1]. In women without an iatrogenic cause for ovarian insufficiency, further differential diagnostic tests such as genetic tests should be performed, but these will not be discussed further here. In most cases, no clear cause will be found [1].

Anti-Müllerian hormone (AMH) is a glycoprotein produced by the granulosa cells of the early antral follicles and correlates with the ovarian reserve. Although AMH concentration in serum is typically reduced in POI, it is not recommended as the sole marker for diagnosis, since normogonadotrophic and regular cycles may still be present when AMH is no longer detectable (Fig. 1).

Fertility in POI

The fertility in patients with POI is severely limited or even abolished. The current data assume a cumulative live birth rate of about 5–10% in affected women. The probability of pregnancy decreases with the duration of amenorrhoea. However, as fertility is often not yet completely eliminated and hormone replacement therapy (HRT) does not usually have a contraceptive effect, appropriate counselling and safe contraception must be provided if it is needed.

However, the probability of conceiving a child must be assumed to be very low. Since patients do not respond to ovarian stimulation or respond very little, assisted reproduction measures cannot significantly improve the prognosis [1, 6].

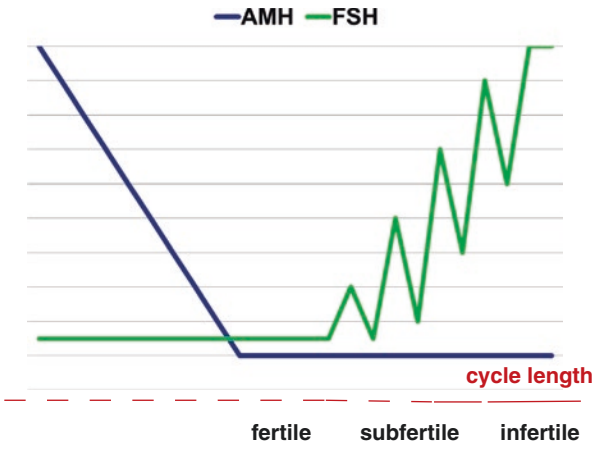


Fig. 1 Concentration course of AMH and FSH and cycle lengths in patients with a low ovarian reserve

POI and Long-Term Consequences

Bone Metabolism

Sex steroids, especially estrogens, are crucial regulators of bone metabolism. A lack of estrogens results in a loss of bone mass and an increased risk of fracture. Osteoporotic fractures in turn lead to a marked reduction in the quality of life, but also in the life expectancy of those affected [7]. In women with POI after chemotherapy there are additional risk factors as a result of direct damage to the bone by treatment or, e.g. in the case of breast cancer, anti-hormonal therapy.

The basic treatment for POI patients is therefore a calcium-rich diet, exercise and vitamin D supplementation.

According to current guidelines, 1000 mg calcium/day and about 1000 IU vitamin D/day should be added to the diet [7].

HRT is one of the forms of therapy for osteoporosis according to guidelines and can prevent a decrease in bone density and therefore reduce the probability of osteoporotic fractures.

Bisphosphonates are established standard drugs in postmenopausal osteoporosis [7]. They are generally not the first choice in POI and should only be prescribed after consultation with an osteologist. If patients are already receiving bisphosphonates in oncological dosages for the treatment of bone metastases, no further specific osteoporosis medication is required.

Bone density measurements should be taken initially in patients with POI and repeated every 1–5 years, depending on the findings and further therapeutic procedures. If bone density is reduced and the risk of fracture is increased, an osteologist should be consulted if necessary [1].

Cardiovascular System

Several studies have demonstrated an increased cardiovascular risk in women with POI/early menopause, including an increase in coronary heart disease, stroke and cardiovascular mortality [1, 8]. HRT is normally preventive. This is mediated by a favourable influence on lipid metabolism, a reduction in the risk of diabetes, a decrease in arteriosclerosis and the regulation of vascular dilatation [1, 6, 9].

Cognition

POI/early menopause is associated with long-term effects on cognitive processes. Increased risks of dementia have been described in women with surgically induced, premature and non-hormone-substituted menopause. The younger the women are

when they enter the menopause, the higher the risk. In contrast, the risk of dementia is not increased in women with POI/early menopause who have HRT up to the age of 50 years [1, 6].

Practical Approach

General

The misinterpretations of the WHI (Women's Health Initiative) study have led to an irrational fear of HRT in both patients and medical professionals. It is now clear, however, that POI/early menopause is associated with an increased risk of cardiovascular disease, osteoporosis and a shortened life expectancy and that HRT is therefore beneficial. The risks of not having HRT must therefore be weighed individually against the risks of giving HRT [10]. However, the oncological (Table 1) and other contraindications for HRT must also be considered.

Hormonal Treatments

The choice of substitution form (Table 2) should replicate ovarian function. This means that standard doses of estrogen therapy should be administered to reduce both acute menopausal symptoms and to prevent long-term consequences of estrogen deficiency (osteoporosis, coronary heart disease, dementia, etc.).

The need for individualized HRT should be noted. Contraindications must be considered (Table 1), the advantages and disadvantages must be explained, and HRT should be re-evaluated annually.

Estradiol appears to have a more beneficial effect than ethinyl estradiol on bone metabolism and the cardiovascular system.

If contraception is required, a variant containing estradiol should be considered. In women with POI/early menopause the estrogen levels drop rapidly during the estrogen-free interphase. Therefore, administration form with continuous estrogen administration should be preferred in cyclical or continuous progestogen therapy. Transdermal estrogen avoids the "first-pass" effect in the liver, reduces activation of coagulation factors and thus the risk of thromboembolic events. Although there is currently not sufficient evidence, it seems biologically and pharmacologically plausible that young women with POI also benefit from the advantages of transdermal HRT [1, 6].

The aim of HRT should be to achieve physiological levels of sex steroids. The recommended doses are summarized in Table 2. In women with an intact uterus, a combined HRT with a progestogen for at least 12–14 days per month is necessary to avoid hyperplasia of the endometrium and to achieve sufficient endometrial

Table 1 Indications and contraindications for hormone replacement therapy (HRT) in different cancer types (modified after [10])

Type of oncological disease	Indication and contraindication for HRT
Breast cancer (estrogen and progesterone sensitive)	HRT contraindicated • HRT should only be offered in distinct exceptions and only in severe and not otherwise treatable impairment of life quality. • Recurrence risk is lowest in estrogen-only treatment.
Healthy BRCA—Mutation carriers, previvors	HRT possible
Endometrial cancer	HRT possible • No change in recurrence risk in estrogen non-sensitive cancer. • Recurrence risk is slightly reduced in estrogen-sensitive cancer when combined HRT is used (estrogen + progesterone). • No change in recurrence risk in estrogen monotherapy (limited data available!).
Cervical cancer	HRT possible • Recurrence risk somewhat reduced in adenocarcinomas if HRT is performed with estrogen + progesterone. • No change in the risk of recurrence in squamous carcinomas.
Ovarian cancer (epithelial and germ cell tumours)	HRT possible
Ovarian cancer (endometrioid and granulosa cell tumours)	HRT relatively contraindicated • Possibly a slight increase in the risk of recurrence.
Non-gynaecological cancer • Haematological malignancies (leukaemia, lymphoma) • Malignant melanoma (localized) • Colorectal cancer • Hepatocellular carcinoma • Renal cancer • Thyroid carcinomas • Pancreatic carcinoma	HRT possible
Non-gynaecological cancer • Cerebral tumour • Malignant melanoma (advanced) • Pulmonary carcinoma • Gastric carcinoma • Bladder carcinoma	HRT relatively contraindicated
Non-gynaecological cancer • Gastric cancer: estrogen, progesterone sensitive— • Bladder cancer: estrogen, progesterone sensitive	HRT contraindicated

Table 2 Hormone preparations for hormone replacement therapy, HRT (formulas are not approved in all countries!)

Estrogen	**Dose**			
	High	**Moderate**	**Low**	**Ultra-low**
Micronized 17ß-estradiol (oral, mg)	4.0	2.0	1.0	0.5
Estradiol valerate (oral, mg)		2.0	1.0	0.5
Transdermal 17ß-estradiol patch (µg)	100	50	25	14 (U.S. only)
Transdermal 17ß-estradiol gel (mg)		ca. 1.0–1.5	ca. 0.5–0.75	
Transdermal 17ß-estradiol spray (mg)		1 0.53		
Conjugated equine estrogens (oral, mg)	1.25/0.9	0.625	0.3/0.45	
Progestogen (the lowest daily dose for endometrial protection is given)	**Sequentially combined HRT**		**Continuously combined HRT**	
	(Ultra) low estrogen dose	**Moderate/high estrogen dose**	**(Ultra) low estrogen dose**	**Moderate/ high estrogen dose**
Dydrogesterone (oral)	5 mg	10 mg	5 mg	5–10 mg
Micronized progesterone (oral/vaginal)	100 mg	200 mg	100 mg	100 mg
Medroxyprogesterone (oral)	5 mg	5–10 mg	2.5 mg	2.5–5 mg
Norethisterone (oral/ transdermal)	1.25 mg	1.25–2.5 mg/170 µg	0.5–1 mg/140–170-250 µg	> 1–2.5 mg
Chlormadinone (oral)	–	–	2 mg	2 mg
Levonorgestrel: Intra-uterine device	–	–	20 µg/24 h for 5 years	

transformation. In women with amenorrhea for more than 1–2 years, continuous combined HRT can also be used. After a hysterectomy, estrogen monotherapy is preferable due to the better side effect profile.

Current studies in postmenopausal women indicate a lower risk of thrombosis with HRT containing metabolically neutral progestogens (micronized progesterone, dydrogesterone) compared to combinations with synthetic progestogens. Although this is only partially transferable to women with POI, it should be considered if women with an increased risk of thrombosis are to be treated. While oestradiol can be applied transdermally without any problems, this is not the case with transdermal micronized progesterone. Therefore, a transdermal application of micronized progesterone does not provide enough endometrial protection.

A hormone-releasing IUD that delivers 20 µg levonorgestrel/day provides contraceptive protection as well as adequate endometrial protection and can be combined with pure estrogen substitution for a period of 5 years [1, 6].

Urogenital symptoms are often not sufficiently improved by systemic therapy. Local estrogens (e.g. estriol) can then be given in addition to systemic HRT.

Non-hormonal Treatments

In addition to conventional HRT, complementary medicine and non-hormonal pharmacotherapy are also available to reduce hot flushes [11].

Cognitive behavioural therapy and hypnosis significantly reduce hot flushes. This also applies to women with breast cancer.

Soy isoflavones can reduce hot flushes. The recommended dose is 50–60 mg isoflavones/day or at least 30 mg genistein extract/day. However, soy isoflavones have no effect on hot flushes in women with breast cancer. Since the safety of isoflavones in women with breast cancer has not been sufficiently investigated, their use in breast cancer patients should be avoided.

Black cohosh was best examined from the field of phytotherapeutics. Black cohosh significantly reduces hot flushes in the peri- and postmenopause, and in premenopausal women with breast cancer (during tamoxifen treatment).

Other oral phytotherapeutic agents used to treat hot flushes are wild yam (Dioscorea), dong quai, evening primrose oil, flaxseed, ginseng, hops, maca, omega-3 fatty acids and a Siberian rhubarb extract ERr 731. However, there is insufficient evidence of efficacy and safety in studies in patients with (hormone-dependent) malignancies.

Acupuncture is part of traditional Chinese medicine and is effective against hot flushes, although no significant reduction of hot flushes could be demonstrated in women with breast cancer. However, in a systematic review of cancer patients, acupuncture was associated with a significant improvement in fatigue, sleep disturbances, pain and quality of life.

A stellate ganglion block is available as a further therapeutic option. This also reduces hot flushes in women with breast cancer.

Besides complementary therapy, various non-hormonal pharmacotherapies can be used.

Antidepressants such as selective serotonin reuptake inhibitors (SSRI) (e.g. paroxetine) and selective serotonin and norepinephrine reuptake inhibitors (SNRI) (e.g. (des-)venlafaxine) significantly reduce menopausal hot flushes in healthy women and women with breast cancer. The starting dose is 10 mg/day for paroxetine (target: 10–20 mg/day) and 37.5 mg/d for venlafaxine (target: 37.5–150 mg/day). In women who are treated with tamoxifen after breast cancer, the SSRI paroxetine should not be administered due to a possible inhibition of the enzyme CYP2D6 and reduction of the effect of tamoxifen, and the SNRI (des-)venlafaxine should be given instead.

The anticonvulsants gabapentin and pregabalin significantly reduce menopausal hot flushes in healthy women and women with breast cancer. The starting dose of gabapentin is 300 mg at night, which can be successively increased to 600 mg at night, then to 300 mg in the morning plus 600 mg at night after 3 days (target: 900–2400 mg/day). The starting dose of pregabalin is 50 mg at night (target: 150–300 mg/day). Possible dose-dependent side effects include drowsiness, headache (often self-limiting after 2–4 weeks).

Diagnostic and Therapeutic Monitoring

Depending on the underlying disease, a general gynaecological check-up should take place at the usual intervals (Fig. 2, Table 3). Patients taking hormone replacement therapy should be seen regularly to assess their climacteric symptoms and adjust the therapy accordingly. This can be done during regular screening appointments. Measurement of serum or saliva levels of estrogen, progesterone or FSH is not recommended [6].

Patients with POI/early menopause should have their bone density measured using DEXA as part of a basic osteological diagnosis. If there are additional risk factors for osteoporosis, such as chemotherapy or estrogen deprivation by aromatase inhibitors, an osteological assessment should be performed. Further controls are carried out every 1–5 years [6, 7] depending on the risk.

There is no specifically increased risk of breast cancer, except in patients with breast cancer or in BRCA gene mutation carriers. Corresponding preventive and screening examinations are therefore carried out according to general recommendations [12].

The cardiovascular risk should be monitored regularly. This includes at least annual monitoring of blood pressure, weight and smoking status and, depending on the risk constellation, further individual tests (e.g. HbA1c, lipid status) [6].

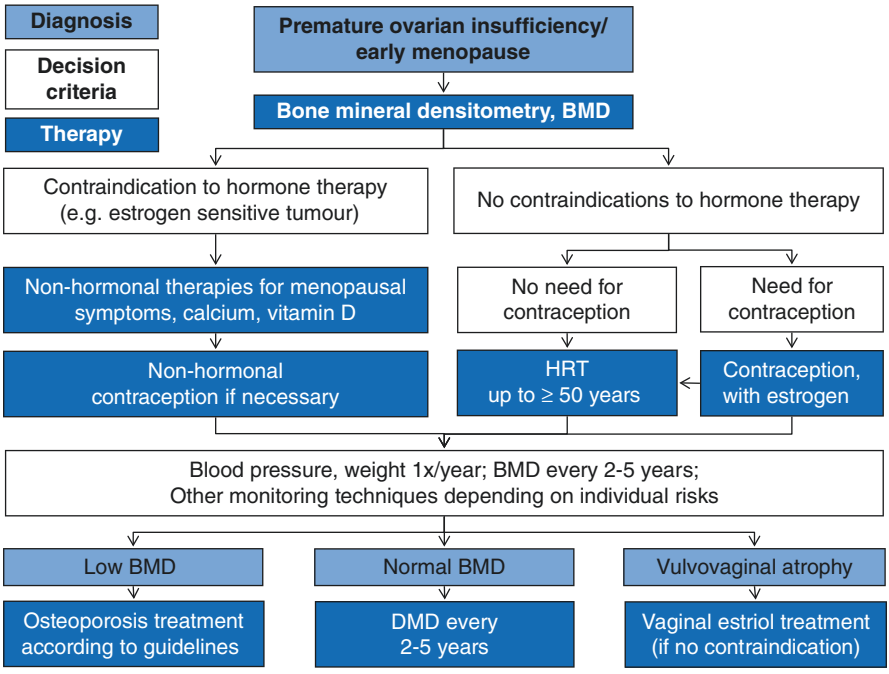


Fig. 2 Treatment decision flowchart of therapy and monitoring in POI/early menopause

Table 3 Monitoring in premature ovarian insufficiency, POI/premature menopause with and without hormone replacement therapy, HRT (modified from [6, 7])

Monitoring procedure	Frequency
General gynaecological check-up and breast examination	Annual check-up. If HRT: additional breast diagnostics if indicated.
Menopausal symptoms evaluation	Part of the annual check-up.
Cardiovascular risk factor evaluation	Annual examination/history of height, weight, blood pressure, smoking status and—depending on the risk constellation—additional examination of lipids, glucose and Hba1c (fasting glucose).
Bone mineral analysis, BMA	Baseline BMA, then: BMA, depending on the bone density, approximately every 2–5 years; support from an osteoporosis specialist may be useful.

References

1. Hamoda H, British Menopause Society and Women's Health Concern. The British Menopause Society and Women's Health Concern recommendations on the management of women with premature ovarian insufficiency. Post Reprod Health. 2017;23:22–35.
2. Coccia ME, Rizzello F, Mariani G, Bulletti C, Palagiano A, Scarselli G. Ovarian surgery for bilateral endometriomas influences age at menopause. Hum Reprod. 2011;26:3000–7.
3. Huong DL, Amoura Z, Duhaut P, Sbai A, Costedoat N, Wechsler B, Piette JC. Risk of ovarian failure and fertility after intravenous cyclophosphamide. A study in 84 patients. J Rheumatol. 2002;29:2571–6.
4. Katsifis GE, Tzioufas AG. Ovarian failure in systemic lupus erythematosus patients treated with pulsed intravenous cyclophosphamide. Lupus. 2004;13:673–8.
5. Oktem O, Kim SS, Selek U, Schatmann G, Urman B. Ovarian and uterine functions in female survivors of childhood cancers. Oncologist. 2018;23:214–24.
6. European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI, Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, Cifkova R, de Muinck Keizer-Schrama S, Hogervorst E, Janse F, Liao L, Vlaisavljevic V, Zillikens C, Vermeulen N. ESHRE guideline: management of women with premature ovarian insufficiency. Hum Reprod. 2016;31:926–37.
7. Dachverband Osteologie e.v. (DVO). Prophylaxe, Diagnostik und Therapie der Osteoporose bei postmenopausalen Frauen und Männern, S3 Leitlinie des Dachverbands der Deutschsprachigen Wissenschaftliche Gesellschaften , 2017, AWMF-Register Nr.: 183–001.
8. Jacobsen BK, Heuch I, Kvale G. Age at natural menopause and all-cause mortality: a 37-year follow-up of 19,731 Norwegian women. Am J Epidemiol. 2003;157:923–9.
9. Elsheikh M, Bird R, Casadei B, Conway GS, Wass JA. The effect of hormone replacement therapy on cardiovascular hemodynamics in women with Turner's syndrome. J Clin Endocrinol Metab. 2000;85:614–8.
10. Deli T, Orosz M, Jakab A. Hormone replacement therapy in cancer survivors - review of the literature. Pathol Oncol Res. 2019. [Epub ahead of print]
11. Stute P, Bürki R, Geissbühler V. Nicht-hormonelle Therapie von menopausalen Hitzewallungen. SGGG Expertenbrief. . https://www.sggg.ch/fachthemen/expertenbriefe/
12. Leitlinienprogramm Onkologie der Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V. (AWMF), Deutschen Krebsgesellschaft e.V. (DKG) und Deutschen Krebshilfe (DKH). S3-Leitlinie Früherkennung, Diagnostik, Therapie und Nachsorge des Mammakarzinoms (Version 4.1, 2018) AWMF-Registernummer: 032-045OL.