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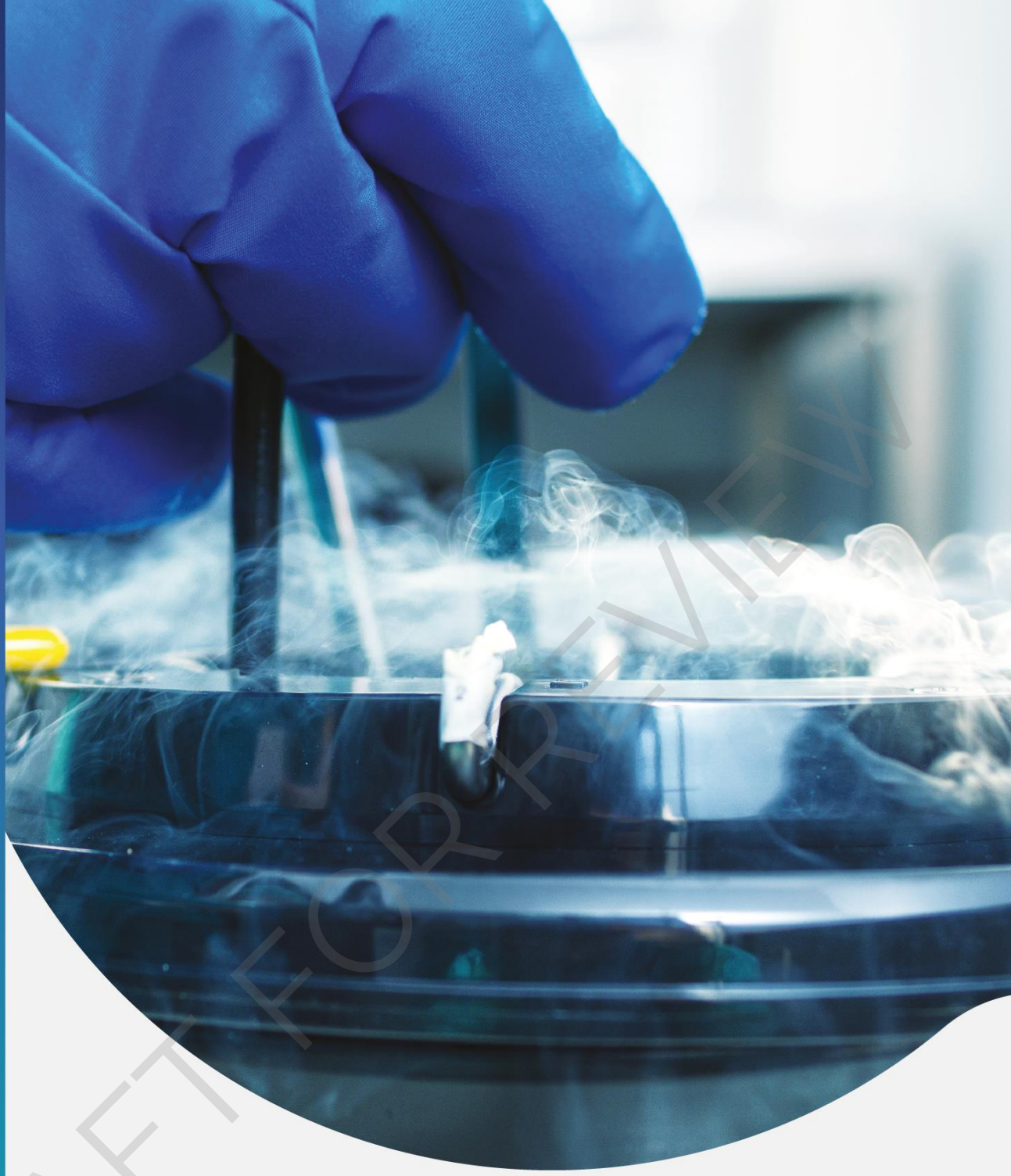

Female Fertility Preservation

Guideline of the European Society of Human Reproduction and Embryology

2020
ESHRE Female Fertility Preservation Guideline Development Group

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Table of Contents

INTRODUCTION 5
Target users of the guideline 5
Guideline scope 5
Patient population 6
Terminology and definitions 6

LIST OF ALL RECOMMENDATIONS 7

PART A: ORGANIZATION AND AVAILABILITY OF FERTILITY PRESERVATION CARE 16
A1. Organisation of care 16
Model of care in FP 16
Multidisciplinary team approach 17
Steps to overcome barriers to fertility preservation 19
Specific care for transgender men 21
Specific care for adolescents 22
Key organisational features for establishing a FP program 23
The need for data collection 23
A2. Legal aspects and availability 26
Cancer patients 26
Patients with benign diseases 26
Transgender men 26
A3. Storage of reproductive material 32

PART B: PATIENT INFORMATION 35
B1. Information needs and provision 35
Patient preferences 37
Decision aids to support patients' decision-making 38
Tools to support clinicians in providing FP information to patients 40
B2. Support and counselling 43
Is there a benefit of psychological support and counselling? 43
Selection of patients for psychological support and counselling 44

PART C: PATIENT SELECTION AND PRE-FP ASSESSMENT 48
C1. Patient selection 48
C2. Gonadotoxic treatments 50
Cancer 51
Benign diseases 57
C3. Ovarian reserve testing 63
Cancer 63
Benign diseases 65
Elective oocyte cryopreservation 69

PART D: FERTILITY PRESERVATION INTERVENTIONS 73
D1. Options for FP 73
D2. Ovarian Stimulation in treatments aimed at FP 75
Preferred protocol 75
Random-start protocol 76
Double stimulation 77
Ovarian stimulation with potentially safer protocols aiming at reducing estrogenic effects and risks 78
Ovarian stimulation for FP in transgender men 79
D3. Oocyte cryopreservation 82
Efficacy of cryopreserved oocytes for fertility preservation 83
Safety and risks 84
Choice of cryopreservation of oocytes versus embryos 85
D4. Oocyte cryopreservation for non-medical reasons 88
Current guidelines on elective oocyte cryopreservation 88
Debates over elective oocyte cryopreservation 88
Issues with elective oocyte cryopreservation 89
Consent and counselling procedures 90
D5. Embryo cryopreservation 92
Embryo stage at time of cryopreservation and method of cryopreservation 92
Effect of age or previous gonadotoxic treatment 93
Efficacy of cryopreserved embryos for fertility preservation and usage rates 93
Choice of cryopreservation of oocytes versus embryos 94
Safety and risks 94
D6. Ovarian tissue cryopreservation 96
Success rates in patients with cancer and benign conditions 96
The ovarian tissue cryopreservation (OTC) procedure 97
The ovarian tissue transplantation (OTT) procedure 98
Impact of ongoing treatments and previous history of chemotherapy on OTC procedure 100
OTC in a combined procedure 101
OTC for other indications 102
Vitrification versus slow-freezing 103
Replacing ovarian tissue: safety concerns 104
D7 In vitro maturation (IVM) 114
In vitro maturation (IVM) after in vivo oocyte aspiration 114
In vitro maturation (IVM) after ex vivo extraction of oocytes from ovarian specimens 116
D8. GnRH agonists 120
Cancer 120
Benign diseases 123
GnRH agonists for Fertility Preservation (all patients) 123
D9. Ovarian transposition 126
Efficacy 126
Complications 127
Technical considerations 128

PART E: AFTER TREATMENT CARE 130
E1. Patient assessment prior to use of stored material 130
Cancer patients 130
Transgender men 134
E2. Obstetric outcomes 137
Effect of chemotherapy 139
Effect of Pelvic radiotherapy 139
Breast cancer 140
Gynaecological cancers 142
Endometrial cancer 142
Other cancers 144
Transgender men 145

PART F: ONGOING DEVELOPMENTS IN FP 149
Technologies involving transplantation into the patient 149
Technologies that do not involve transplantation 151
Treatments to prevent gonadotoxic-induced POI 153

ANNEX 1: GUIDELINE GROUP 159
Declarations of interest 160
ANNEX 2: ABBREVIATIONS 161
ANNEX 3: RESEARCH RECOMMENDATIONS 162
ANNEX 4: METHODOLOGY 163
ANNEX 5: STAKEHOLDER REVIEW 166
ANNEX 6 : SURVEY ON THE LEGAL ASPECTS AND STORAGE: METHODOLOGY 167
ANNEX 7: LITERATURE STUDY REPORT SEPARATE DOCUMENT
ANNEX 8: EVIDENCE TABLES SEPARATE DOCUMENT

Introduction

This is the first ESHRE evidence-based guideline on female fertility preservation.

The guideline was developed according to a well-documented methodology, universal to ESHRE guidelines and described in the Manual for ESHRE guideline development (www.eshre.eu). Details on the methodology of the current guideline are outlined in Annex 4.

The guideline development group (GDG) was composed of (previous) members of the coordination of the SIG Fertility Preservation and Quality and safety in ART, with addition of experts in the field, including a psychologist, oncologist, ethicist and 2 patient representatives. The members of the guideline development group are listed in Annex 1.

Target users of the guideline

The guideline is aimed at healthcare professionals who have direct contact with, and make decisions concerning the care of women scheduled to undergo gonadotoxic treatments or women considering fertility preservation for other reasons. This includes, but is not limited to, reproductive medicine specialists, endocrinologists, oncologists and oncological surgeons and gynaecologists, paramedical and reproductive biologists (including embryologists), and geneticists.

For the benefit of patient education and shared decision-making, a patient version of this guideline will be developed.

Guideline scope

The field of fertility preservation has grown hugely in the last two decades, driven by the increasing recognition of the importance of potential loss of fertility as a very important effect of the treatment of cancer and other serious diseases, and the development of the enabling technologies of oocyte vitrification and ovarian tissue cryopreservation for subsequent autografting. This has led to the widespread (though uneven) provision of fertility preservation for many women and indeed young girls, in parallel to the long-established option of sperm cryopreservation for postpubertal men. The very rapid development of this field in clinical practice, yet with limited data on outcomes, has led to the need for the evaluation of the underpinning evidence base and the development of guidelines to assist practitioners in its safe and effective implementation.

In this document ESHRE seeks to provide an evidence-based guideline for the provision of fertility preservation services. The remit and scope are to evaluate all aspects of this topic in relation to its application for adult women and specifically to include its relevance to transgender men. Its application for prepubertal girls is not included comprehensively, although the application of ovarian tissue cryopreservation for this patient group is alluded to in the relevant section. Additionally, this guideline seeks to be inclusive regarding indications for fertility preservation. Thus, in addition to cancer diagnoses as the most common indication for FP, its application in other serious diseases where treatment with cytotoxic agents is necessary is also considered, as are emerging indications in other metabolic, genetic and chromosomal conditions such as Turner Syndrome. Women are increasingly opting to cryopreserve oocytes for non-medical indications, a process often called “social egg freezing”. The medical and ethical aspects of this indication are also included in this guideline. In many of these conditions the evidence base remains limited, and we have sought to highlight particular areas where further research is needed.

Patient population

The current document outlines FP options for 4 populations:

- Women diagnosed with cancer undergoing anticancer treatments
- Women with benign diseases undergoing gonadotoxic treatments and with conditions that mean they will loose their fertility prematurely, eg Turner syndrome
- Transgender patients (assigned females at birth)
- Women requesting elective oocyte cryopreservation

In any of these 4 populations, the guideline restricts the recommendations to adults and adolescent (post pubertal) patients that are considered healthy enough and suitable to undergo FP procedures.

Specific issues on adolescents are covered where relevant throughout the guideline.

Terminology and definitions

For consistency and clarity, the guideline group decided on the terminology used throughout this document, where relevant in line with published terminologies.

Suggested terminology	Instead of
FP in cancer patients	oncofertility
MAR (medically assisted reproduction)	ART (assisted reproductive technology)
Oocyte cryopreservation	Egg freezing
Embryo cryopreservation	Embryo freezing
Transgender men	Transgenders, transgender people, assigned females at birth
Oocyte pick-up (OPU)	Oocyte retrieval, oocyte collection
AYA (adolescents and young adults)	
TAYA (transgender adolescents and young adults)	

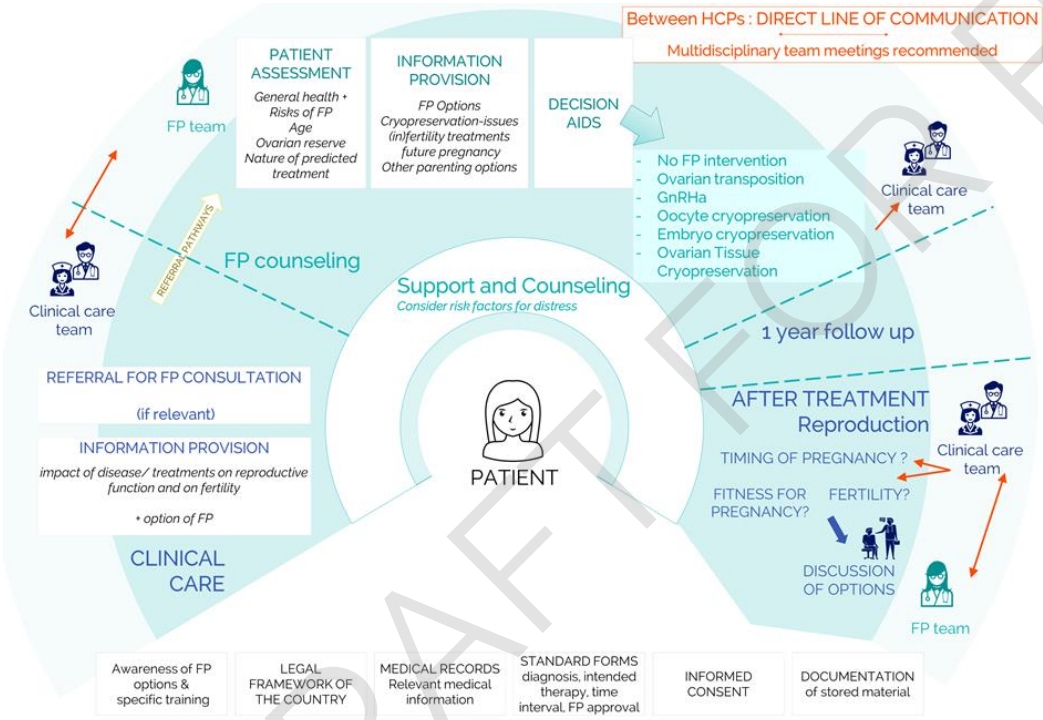
With regards to the healthcare professionals involved, the guideline uses "clinical care team" to indicate the team (whatever the composition) organizing and caring for the patients' primary condition. Some examples include the oncology team, the rheumatology team, the endometriosis team, the transgender identity team, etc.

Fertility and fertility preservation (FP) are considered by a team of clinicians and associated healthcare professionals at the fertility clinic (hereafter referred to as the "FP team")

A list of abbreviations used in this document is included in Annex 2.

List of all recommendations

Nr	Recommendation	Strength	Quality of evidence	Justification	Remarks
PART A: ORGANISATION AND AVAILABILITY OF FERTILITY PRESERVATION CARE					
How should the care for women undergoing fertility preservation be organized?					



PART B: PATIENT INFORMATION

Which information needs to be provided to women at risk of infertility?

1	Clinicians should provide information to patients regarding 1) impact of cancer, other diseases and their treatments on reproductive function; 2) impact of cancer, other diseases and their treatment on fertility, 3) fertility preservation options; 4) cryopreservation related issues after FP, 5) infertility and fertility treatments; 6) pregnancy after cancer; and 7) other childbearing and parenting options.	STRONG	⊕⊕○○
2	Information provided should be specific to the patients' needs.	GPP	
3	Age-specific information and counselling should be provided for adolescents and young adults.	GPP	

How should information on fertility preservation options be provided to patients?

4	It is recommended to provide decision aids to patients who are considering FP.	STRONG	⊕⊕○○
5	Healthcare professionals may consider the use of a checklist for a better provision of information to patients.	WEAK	⊕○○○

Is there a benefit of psychological support and counselling, and are there particular groups that would benefit from it?

6	It is recommended that patients are offered psychological support and counselling when dealing with FP decisions, although the extent of the clinical benefit has not been studied.	STRONG	⊕○○○
7	Clinicians may consider referring FP patients who present risk factors for psychological distress for psychological support and counselling.	WEAK	⊕○○○

PART C: PATIENT SELECTION AND PRE-FP ASSESSMENT

Which criteria can be used to select patients for fertility preservation?

8	Patients require an individual assessment of the need and suitability of FP.	GPP	
9	A multidisciplinary team is recommended to have an accurate assessment of risks.	GPP	

Which factors should be taken into account when estimating the individual risk of gonadotoxicity for a certain patient?			
10	The risk of gonadotoxicity should be assessed in all patients undergoing anticancer treatments	GPP	
11	To estimate the individual risk of gonadotoxicity, the characteristics of the proposed treatment, the patient and the disease should be considered	STRONG	⊕⊕◯◯
Is it relevant to do ovarian reserve testing, and for whom?			
12	Pre-treatment ovarian function, in particular through AMH levels, in premenopausal women with a diagnosis of breast cancer or haematological malignancy is a relevant predictor of post-treatment recovery of ovarian function (evaluated as recovery of menses).	STRONG	⊕⊕◯◯
13	For patients in whom you want to know fertility status, the value of pre-treatment AMH levels for predicting post-treatment fertility is unclear.	WEAK	⊕◯◯◯
14	Age, pre-treatment AMH levels, as well as proposed gonadotoxic treatment type and dose, should be taken into consideration when estimating the risk of post-treatment POI.	STRONG	⊕◯◯◯
15	Pre-treatment ovarian reserve testing in women with malignancies (other than breast or haematological cancer) is likely to be of high relevance, based on the indirect evidence from breast and haematological cancers.	WEAK	⊕◯◯◯
16	The relevance of ovarian reserve testing to help guide fertility preservation options or treatment decisions in SLE patients is low.	WEAK	⊕◯◯◯
17	The relevance of ovarian testing to help guide fertility preservation options or treatment decisions in endometriosis patients remains inconclusive.	WEAK	⊕◯◯◯
18	In patients with endometriosis, the involvement of the ovaries and the radicality of surgery influence ovarian reserve as measured by AMH levels, however their effect on future fertility is unclear.	GPP	
19	For women with overt POI, fertility preservation is not recommended.	GPP	
20	For women with reduced ovarian reserve (Bologna criteria, AMH 0.5-1.1ng/ml), advise needs to be individualized and the value of FP is unclear.	GPP	

21	For predicting high and low response to ovarian stimulation, use of either antral follicle count (AFC) or anti-Müllerian hormone (AMH) is recommended over other ovarian reserve tests.	STRONG	⊕⊕◯◯

PART D: FERTILITY PRESERVATION INTERVENTIONS

How should ovarian stimulation be performed in cancer patients undergoing FP treatment?

22	For ovarian stimulation in women seeking fertility preservation for medical reasons the GnRH antagonist protocol is recommended for its feasibility in urgent situations, short time and safety reasons.	STRONG	⊕◯◯◯
23	For patients requiring ovarian stimulation where there is a lack of urgency, the use of a long protocol may be appropriate.	WEAK	⊕◯◯◯
24	In urgent fertility preservation cycles, random-start ovarian stimulation is an important option.	WEAK	⊕⊕◯◯
25	Double stimulation can be considered for urgent fertility preservation cycles.	WEAK	⊕⊕◯◯
26	In ovarian stimulation for fertility preservation in estrogen-sensitive diseases the concomitant use of anti-estrogen therapy, such as letrozole, is probably recommended.	GPP	
27	For ovarian stimulation in transgender men aiming at oocyte cryopreservation, GnRH antagonist protocols have been reported as feasible and with numbers of oocytes retrieved comparable to those obtained in cisgender women when individuals have stopped previous treatment with testosterone.	WEAK	⊕◯◯◯
28	Addition of letrozole to the antagonist protocol may enhance treatment adherence for transgender men by reducing estrogenic symptoms.	GPP	

Is oocyte cryopreservation effective and safe for FP?

29	Oocyte cryopreservation should be offered as an established option for fertility preservation.	STRONG	⊕⊕◯◯
30	Women with a partner should be offered the option to cryopreserve unfertilized oocytes or to split the oocytes to attempt both embryo and oocyte cryopreservation.	GPP	
31	Women should be informed of accurate, centre-specific expertise and live birth rates. They should also be informed that success rates after	GPP	

	cryopreservation of oocytes at the time of a cancer diagnosis may be lower than in women without cancer.		
32	Women considering elective oocyte cryopreservation should be fully informed regarding the success rates, risks, benefits, costs and the possible long-term consequences, both in terms of physical and psychological health.	STRONG	⊕○○○
33	Suitability should be determined on a case-by-case basis.	GPP	
Is embryo cryopreservation effective and safe for fertility preservation?			
34	Embryo cryopreservation is an established option for fertility preservation.	STRONG	⊕⊕○○
35	Women should be informed about the risk of losing reproductive autonomy and possible issues with ownership of stored embryos.	GPP	
36	Women should be informed of accurate, centre-specific expertise and live birth rates. They should also be informed that success rates after cryopreservation of embryos at the time of a cancer diagnosis may be lower than in women without cancer.	GPP	
Should ovarian tissue cryopreservation (OTC) versus no intervention be used for FP?			
37	OTC is an effective method for ovarian function and fertility preservation. It is recommended to offer OTC in patients undergoing moderate/high risk gonadotoxic treatment where oocyte/embryo cryopreservation is not feasible, or at patient preference.	STRONG	⊕⊕○○
38	OTC should probably not be offered to patients with low ovarian reserve (AMH<0.4ng/ml and AFC<5) or aged above 36 years considering the unfavourable risk/benefit.	WEAK	⊕○○○
39	The GDG recommends that OTC is considered to be an innovative method for ovarian function and fertility preservation in post-pubertal women.	GPP	
40	Young patients who have already received low gonadotoxic treatment or a previous course of chemotherapy, can be offered OTC as FP option.	WEAK	⊕○○○
41	Ovarian stimulation can be performed immediately after OTC.	WEAK	⊕○○○
42	OTC at the time of oocyte pick-up after ovarian stimulation should not be performed unless in a research context.	RESEARCH ONLY	
43	Ovarian transposition can be performed at the same time as OTC in patients who will receive pelvic irradiation.	GPP	

44	OTC is not recommended as primary FP procedure in transgender men but can be proposed as an experimental option when ovaries are removed during gender reassignment surgery.	GPP	
45	Ovarian tissue transplantation (OTT) can be considered in patients with POI-associated genetic and chromosomal disorders but requires genetic counselling and should be performed within a research protocol.	RESEARCH ONLY	
Should vitrification versus slow-freezing be used for ovarian tissue cryopreservation for FP?			
46	The slow-freezing protocol for OTC is well-established and considered as standard.	STRONG	⊕○○○
47	Vitrification of ovarian tissue should only be offered within a research program.	RESEARCH ONLY	
Which safety issues should be considered when replacing ovarian tissue?			
48	A standard laparoscopy procedure for OTT is considered safe without causing additional surgical risk.	STRONG	⊕⊕○○
49	OTT at the orthotopic site is recommended to restore fertility	STRONG	⊕⊕○○
50	The decision to perform OTT in oncological patients requires a multidisciplinary approach.	GPP	
51	It is recommended to evaluate the presence of residual neoplastic cells in the ovarian cortex (and in the residual medulla when available) using appropriate techniques in all cancer survivors before OTT.	STRONG	⊕○○○
52	OTT is not recommended in cases where the ovary is involved in the malignancy.	STRONG	⊕○○○
53	Hormone-sensitive tumours such as endometrial and breast cancer are not a contraindication for OTT and pregnancy after complete remission of the disease.	STRONG	⊕⊕○○
54	There appears to be no increased risk of congenital abnormalities for children born after OTT.	WEAK	⊕○○○
55	Long-term risks in human are considered to be low but a long-term follow-up of patients after OTT is probably recommended.	GPP	
56	OTT can be offered in BRCA patients, but the ovarian tissue must be completely removed after subsequent pregnancy.	WEAK	⊕○○○

Should in vitro maturation (IVM) be used for FP?			
57	IVM should be regarded as an innovative FP procedure.	STRONG	⊕○○○
58	IVM requires specific expertise and should only be performed when oocyte cryopreservation is required but ovarian stimulation not feasible.	GPP	
59	IVM after ex vivo extraction is considered an experimental procedure	WEAK	⊕○○○
Should GnRH agonists versus no treatment be used for ovarian protection in patients undergoing gonadotoxic treatment?			
60	GnRH agonists during chemotherapy should be offered as an option for ovarian function protection in premenopausal breast cancer patients receiving chemotherapy; however, limited evidence exists on their protective effect on the ovarian reserve and the potential for future pregnancies.	STRONG	⊕⊕⊕⊕
61	In women with breast cancer, GnRH agonists during chemotherapy should not be considered an option for fertility preservation instead of cryopreservation techniques.	STRONG	⊕⊕⊕○
62	In malignancies other than breast cancer, GnRH agonists should not be offered as an option for ovarian function protection and fertility preservation.	STRONG	⊕○○○
63	GnRH agonists during chemotherapy may be considered as an option for ovarian function protection in premenopausal patients with autoimmune diseases receiving cyclophosphamide. However, it should be acknowledged that limited data are available in this setting.	WEAK	⊕⊕○○
64	GnRH agonists should not be considered an equivalent or alternative option for fertility preservation but can be offered after cryopreservation techniques or when they are not possible.	GPP	
Should transposition of ovaries versus no treatment be used for ovarian protection?			
65	Where pelvic radiotherapy without chemotherapy is planned, women may be offered ovarian transposition with the aim to prevent premature ovarian insufficiency.	WEAK	⊕⊕○○
66	Women with reduced ovarian reserve and women at risk of having ovarian metastases are inappropriate candidates for ovarian transposition.	GPP	

PART E: AFTER TREATMENT CARE

How should patients be re-assessed before use of stored material?

67	Before the use of stored material, fitness for pregnancy should be thoroughly assessed, taking into account treatment late effects, the age of the patient and the interval since treatment.	STRONG	⊕○○○
68	The need for psychological counselling, pre-conception counselling and fertility treatment counselling should be considered for all patients. Local guidelines for counselling should be followed.	GPP	

What is the effect of previous gonadotoxic treatments and underlying conditions on obstetric outcomes?

69	Preconception counselling and appropriate obstetric monitoring is recommended in women intending to become pregnant after anticancer treatments.	STRONG	⊕⊕⊕○
70	An interval of at least 1 year following chemotherapy completion should be considered before attempting a pregnancy in order to reduce the risk of pregnancy complications.	STRONG	⊕○○○
71	Radiotherapy to a field that included the uterus increases the risk of pregnancy complications; this risk is age and dose dependent. These pregnancies should be treated as high risk and managed in a centre with advanced maternity services.	STRONG	⊕○○○
72	After completion of the recommended treatment, pregnancy is safe in women who have survived breast cancer. This is independent of estrogen receptor status of the tumour.	STRONG	⊕⊕○○
73	Pregnancy after treatment for breast cancer should be closely monitored, as there is an increased risk of preterm birth and low birth weight. Patients should be informed about these risks.	STRONG	⊕⊕⊕○
74	Reliable non-hormonal contraception is mandatory during tamoxifen treatment. It is recommended to stop tamoxifen for at least 3 months before attempting pregnancy.	GPP	
75	Women with endometrial cancer, should be followed up for high-risk pregnancy and monitored by an oncologist, due to the risk of relapse.	STRONG	⊕○○○
76	The risk of preterm birth is increased after treatment for early cervical cancer and these pregnancies should be treated as high risk and managed in a centre with advanced maternity services.	STRONG	⊕⊕○○

[15]

77	Women previously treated for cancer require individual assessment of their obstetric risks and potential additional obstetric surveillance.	STRONG	⊕○○○
78	Healthcare professionals should have a high level of awareness of the risk of depression and increased dysphoria during and after pregnancy care for transgender men.	WEAK	⊕○○○

3

PART A: Organization and availability of fertility preservation (FP) care

A1. Organisation of care

NARRATIVE QUESTION: HOW SHOULD THE CARE FOR WOMEN UNDERGOING FERTILITY PRESERVATION (FP) BE ORGANIZED?

This guideline focuses on several distinct groups of patients, including patients diagnosed with cancer undergoing gonadotoxic treatments, patients with benign diseases undergoing gonadotoxic treatments or those with a genetic condition predisposing to premature ovarian insufficiency, transgender men (assigned females at birth), and women requesting elective oocyte cryopreservation. Despite differences between these patients, FP care should be organized in an optimal way to accommodate all of them, taking into consideration the appropriate local legal context.

With regards to organization of care, the most important difference between the patient types lies in the urgency of FP treatment. For instance, in oncology patients, FP treatment is often urgent (not to cause a delay in starting cancer treatments), and this requires different communication and referral pathways, compared to other indications where FP treatment can be discussed with the patient, fully considered and scheduled conveniently.

In order to improve the quality of health care for patients undergoing FP, a multi-level approach is necessary (Ferlie and Shortell, 2001), addressing issues specific to:

1. The patient (and his/her partner and/or parents),
2. Professionals,
3. Organization (clinic, hospital),
4. Policy makers and general population.

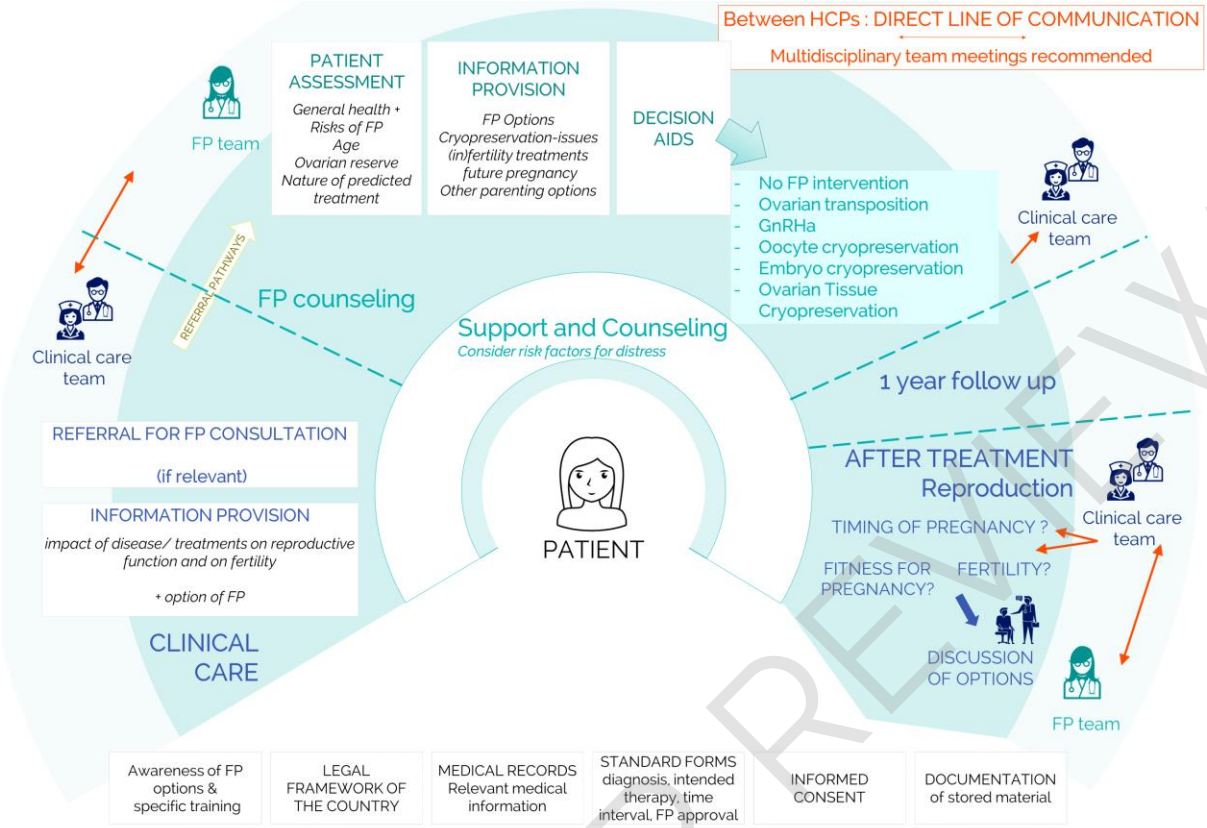
All these issues require consideration when developing and optimising the organization of care in FP.

Model of care in FP

The current chapter on organisation of care and the chapter on information provision combined picture an overview of how the care for a patient eligible for FP can be organised. Figure 1 provides a schematic presentation of the most relevant information (Figure 1).

[17]

Figure 1 Model of care for patients eligible for fertility preservation



Multidisciplinary team approach

Women eligible for FP interventions will be managed by different clinical care teams. The clinical care team consists of the oncology team for cancer patients, a rheumatologist, gynaecologist, endocrinologist, haematologist, or another specialist physician for women with benign diseases, and the gender assignment team for transgender men. Women requesting elective oocyte cryopreservation may directly approach the FP team, or be referred by their general practitioner or gynaecologist.

The FP team should consist of fertility specialists, and embryologists, but should also include a psychologist or counsellor. A dedicated psychologist or counsellor improves communication between doctors and patients and helps to meet emotional needs (Razzano *et al.*, 2014).

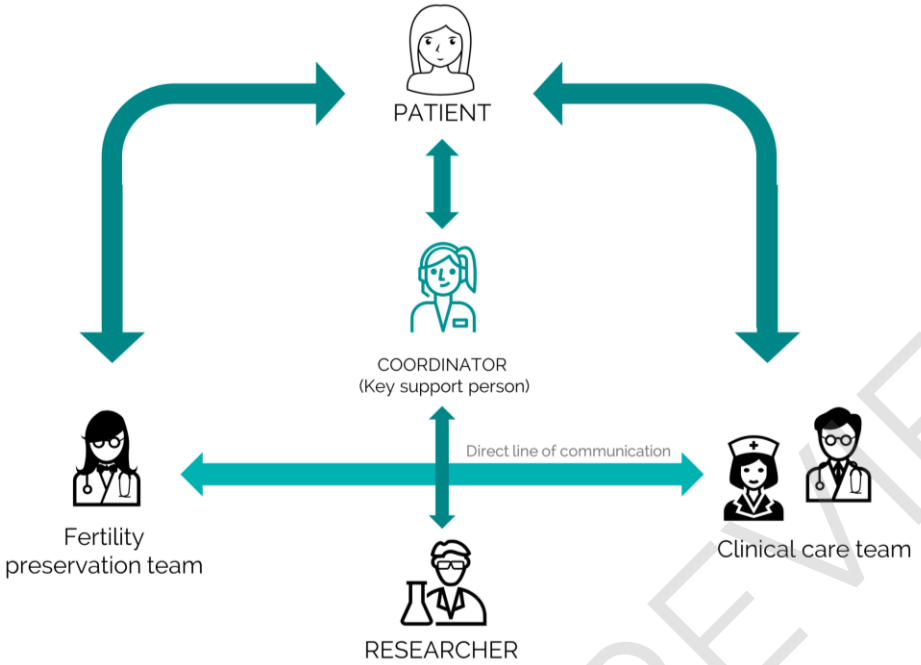
It is critical to ensure there is a direct communication pathway with the FP team. Including a key support person (termed "coordinator") in the clinical care team can be considered to support the patients and ensure they are offered timely referral to the FP team (see Figure 2). This person can also be responsible for communication regarding clinical trials.

Whenever FP treatment is considered for adolescents, the inclusion of a paediatrician in the clinical care or FP team is recommended (more information in the section on adolescents below).

The clinical care team is usually responsible for referral to the FP team. For timely and appropriate referral, awareness of the different FP options with their benefits and limitations is essential. (see section on Oncologists' awareness of FP options). As FP is a rapidly developing area and new methods or strategies are continuously being developed, there is a need to share information and have ongoing communication between the teams.

[18]

Figure 2 The multidisciplinary team and the role of the "coordinator"



Conclusion

There should be agreement on who is responsible for the different issues:

- Referral: who is responsible, and how, what information should be included?
- Standard forms including diagnosis, intended therapy, time interval are recommended.
- Check if FP counselling has been offered and has taken place.
- FP treatment: a member of the FP team should discuss any FP treatment with the clinical care team before starting treatment.

Registration:

- All relevant medical information should be documented in the patients' medical records.
- All patients undergoing FP should have been counselled about the legal and financial consequences and must have given written informed consent.
- Accurate documentation, especially about the gametes/embryos/tissue stored, is essential as it may be in storage for many years.

There should be a direct link between the clinical care team and the FP team, preferably in multidisciplinary team meetings.

There should be a key individual (the 'coordinator') in clinical care teams to support patients of reproductive age to see a member of the FP team.

Psychological support/counselling should be available to all patients considering FP. Specific support for particular patient groups may be required, eg adolescents with their parents

Steps to overcome barriers to fertility preservation.

Ideally, all patients of reproductive age scheduled to undergo gonadotoxic treatment should be referred to the FP team for FP counselling and, if relevant, treatment. Similarly, transgender men should be informed on fertility issues and FP options before starting hormonal treatments.

It is unclear how many patients should receive FP counselling and/or treatment, and how many patients are counselled by the FP team, but there are some clear barriers that prevent patients from accessing appropriate FP counselling and FP treatment.

The barriers can be summarized as:

- Limited public awareness of fertility and FP
- Limited oncologists' awareness of FP options
- Lack of referral pathways, mainly described in oncology patients
- Unavailability of every FP procedures
- Lack of specific care for transgender men and awareness of FP options

These barriers are discussed in more details below

Improving public awareness of fertility and of factors that may have negative effects on it

Recent initiatives by the British Fertility Society and the patient organization Fertility Europe in collaboration with ESHRE have highlighted the current limited understanding of fertility and particularly how it changes with female age.

In 2016, "The fertility education initiative" was launched by the British Fertility Society. (https://www.britishfertilitysociety.org.uk/fei/) to address this. It is a programme of work dedicated to improving knowledge of fertility and reproductive health, set up in response to a growing debate and concern amongst health and education professionals, about the lack of knowledge about age related decline in fertility.

> To increase awareness in the general population and policy makers, education about fertility, reproductive lifespan and changes with age (both male and female) should be standard in school, as part of reproductive health education on contraception/family building/relationships.

Oncologists' awareness of FP options

With increasing prevalence of cancer in young women, and increasing numbers of survivors, it has become increasingly important to pay attention to the late side effects of cancer treatment. In young women the long-term quality of life is often diminished by concerns about their future fertility and pregnancy. Important international guidelines underline the significance of counselling every young women or girl and/or her parents before treatment about the impact of gonadotoxic treatment on later fertility and the possibilities regarding FP (Anazodo *et al.*, 2019) however, the quality of these guidelines can be improved (Baysal *et al.*, 2018). These issues are often not addressed, and patients are often not referred for counselling about FP, with referral rates of 9.8% (Bastings *et al.*, 2014), 10.7% (Korkidakis *et al.*, 2019) and 47% (Quinn *et al.*, 2011b) identified. In a systematic review by Goossens et al, the proportion of patients receiving information about cancer-related infertility varied from 0% - 85% (Goossens *et al.*, 2014).

There is a need for more awareness at the professional level. There appears to be a lack of knowledge of cancer related infertility and fertility preservation options in oncologists (Yee *et al.*, 2012, Miller *et al.*, 2017, van den Berg *et al.*, 2019). Gaps in knowledge of health care professionals were found in relation to existing guidelines, FP procedures, costs, fertility facilities and specialists and educational material for patients (Vindrola-Padros *et al.*, 2017). Knowledge about the options available for girls and young women are even less well known (Vindrola-Padros *et al.*, 2017).

At the time of a cancer diagnosis, patients are often overwhelmed and may have difficulties in thinking about other issues, such as future fertility (Niemasik *et al.*, 2012). They also are afraid to negatively influence their prognosis by postponing cancer therapy. Women reported that their physicians brought up the risk of recurrence of cancer in hormone positive tumours as reasons for not being prepared to delay cancer treatment. Patients also reported that physicians often assumed that women who already have one or more children do not wish to retain their fertility for the future. Furthermore, some women without children and without a partner had the impression from health care professionals that they were unsuitable for FP.

Some professionals are reluctant to start a discussion about future fertility with adolescents and their parents, because they perceive these fertility conversations as potentially embarrassing. But adolescents may also feel embarrassed to talk about future fertility, especially when their parents are included in the discussion. Teenagers and young adults expressed a wish to have a choice in who should be included in these discussions (Crawshaw *et al.*, 2009).

Second phase of fertility preservation (after cancer treatment):

While much of this guideline focusses on fertility preservation before cancer treatment, it is important that patients as well as professionals are aware of the fact that it is important to discuss future (in)fertility after cancer treatment. This may involve referral to a reproductive medicine centre for discussion regarding assessment, provision of individualized advice regarding natural fertility, and where appropriate treatment with or without the use of their stored material. This may also include the possibility of FP after treatment if pregnancy is not yet desired.

Conclusion

> Fertility preservation should be included in basic general medical education, and in the training of medical, surgical, radiological, and gynaecological oncologists, rheumatologists, gynaecologists, endocrinologists, haematologists and other professionals who might start treatment in women with benign diseases that may have a negative impact on fertility. Professionals working in Reproductive Medicine should maintain up to date knowledge and skills in this field.

> Clinicians and nurses in related specialties, particularly in oncology, should follow educational programs regarding FP on a regular basis. National societies for Oncology and Obstetrics & Gynaecology should work together in developing training materials and curricula as well as adopting FP guidelines in national protocols and guidelines.

> Specific training programs should be developed for counselling adolescents and their parents/carers.

Improvement of referral pathways

Problems with service delivery and transitioning between clinical care and fertility services hinder the FP decision-making. Often there is no fertility preservation program available, or no referral policy in oncology units (Panagiotopoulou *et al.*, 2018).

In the literature long waiting lists to see a fertility specialist are reported, with appointment delays until after chemotherapy had already started (Corney and Swinglehurst, 2014). Many studies reported poor coordination of care between different medical centres and patients felt they were being pushed from provider to provider with no-one helping them to make decisions (Gorman *et al.*, 2012, Yee *et al.*, 2012). Importantly, an educational intervention among nurses increases rates of discussion, referral and documentation (Quinn *et al.*, 2019). Fertility preservation counselling by a fertility specialist results in less decisional regret and a better quality of life (Letourneau *et al.*, 2012, Skaczkowski *et al.*, 2018).

It is also important to develop protocols for the care for these patients regarding future fertility after cancer treatment. It may be appropriate to make a referral to a fertility specialist to discuss (future) pregnancy options, fertility preservation, oocyte donation, etc, one year after cancer treatment although this will be dependent on the age and situation of the patient. This is further discussed in the section on patient information.

Costs and financial reimbursement are an important barrier for patients worldwide restricting FP as an option. This has been illustrated in surveys and systematic reviews (Jones *et al.*, 2017, Rashedi *et al.*, 2018).

Conclusion

> Oncologists and specialists in other relevant specialties should consider the potential need for FP in all women of reproductive age, including adolescents.

> Urgent referral pathways need to be established allowing patients to be seen by a member of the FP team within 24-48 hours after referral.

> Referral criteria should be set up to enable this in regional arrangements between care teams looking after patients possibly requiring FP and fertility specialists: this should include the names of institutes that deliver FP as well as their contact persons and contact details. These FP clinics should have awareness of the specific needs of all patient groups, including transmen. Information about financial costs should be provided. These checklists should be part of a standard operating procedure (SOP).

> A follow-up appointment with a FP doctor is recommended approximately 1 year after treatment for adults, and at an appropriate age for younger adolescents

Availability of different FP procedures

Embryo cryopreservation and oocyte vitrification are widely performed worldwide, whereas the availability of ovarian tissue cryostorage is more limited. Several reports have demonstrated the feasibility of harvesting the ovarian cortex in one clinic and then transporting it to another centre to be frozen. This is discussed in more detail in chapter D6. Ovarian tissue cryopreservation but requires formal agreements between clinics/tissue establishments and specific and detailed procedures.

Women without a uterus or receiving high doses of pelvic irradiation will need specific counselling regarding surrogacy in the future. This may not be available at the referral clinic; thus, professionals should be aware that these women might need onward referral to discuss this.

Conclusion

> Embryo and oocyte vitrification can be performed in most IVF centres. Due to the relatively low number of procedures required, the technique of ovarian tissue cryopreservation should be concentrated in a few centres with appropriate expertise.

Specific care for transgender men

Aspects of reproductive function are major contributors to gender dysphoria, and the endocrine and surgical treatment of transgender people will often compromise their fertility. However, the desire for parenthood is prevalent among transgender people, and thus there is an important need for FP. A systematic review demonstrated that 1/3 to 2/3 of transgender adolescents and young adults (TAYAs) desire having children sometime in their lifetime (Baram *et al.*, 2019). Transgender men bring specific issues to the provision of FP. There is a need for a trans-friendly clinic

environment: referral forms should be designed to allow patients an opportunity to indicate what pronouns and names they prefer, providers should be trained to use gender-neutral languages, and there may be difficulties with the conventional transvaginal approach to monitoring and oocyte pick-up (Armuand *et al.*, 2017).Transgender people and their partners have predominantly negative interactions with fertility service providers when they access or attempt to access services at fertility clinics (James-Abra *et al.*, 2015). They often are treated with disrespect and discrimination: there are reports of patients being denied access to FP counselling and services by clinical staff after disclosing their trans gender identities (Eisenberg *et al.*, 2020). The majority of health care providers do not have enough knowledge about FP options for TAYAs. FP counselling for TAYAs is difficult because of the lack of evidence about the effects of gender-affirming hormone treatment on reproduction. Therefore, most TAYAs lack awareness of the FP options, costs, invasiveness of the procedures and the potential psychological impact of going through the process (Baram *et al.*, 2019).

The literature suggests that FP counselling should begin prior to undergoing gender-affirming hormone treatment and that FP counselling and support services should be the standard of care (Baram *et al.*, 2019). The Endocrine Society recommended in 2017 against puberty blocking followed by gender affirming hormone treatment of prepubertal children. However, they stated that clinicians should inform pubertal children and adolescents seeking gender affirming treatment of the options of fertility preservation (Hembree *et al.*, 2017). In relation to ovarian stimulation, this is discussed further in section D2. Ovarian Stimulation in treatments aimed at FP.

Conclusion

FP counselling and support services should be standard of care for transgender adolescents and young adults. While it would seem most appropriate to offer FP before starting gender-affirming hormone treatment, it is recognized that this may not be possible, and FP remains a possibility after starting gender-affirming hormone treatment.

Health care professionals in transgender care should be educated about FP options, and similarly staff working in reproductive medicine need to be aware of the need for appropriate care of transgender men, with the development of specific approaches and protocols.

Specific care for adolescents

Adolescents are a special case, and it is as important to include assessment of psychological as physical maturity. One review that included 16 papers on 14 studies on FP in children, adolescents and young adults referred that health care professionals reported embarrassment when discussing FP with children and young people (Vindrola-Padros *et al.*, 2017). Decisions on whether to discuss FO with young patients were depended on the knowledge and sense of comfort of the clinician, the sexual maturity and prognosis of the patient, parent involvement and availability of educational materials. Ten studies included in the review highlighted issues regarding the role of parents in FP discussions. Specifically, the presence of the parents was judged as evoking embarrassment in the young patient and that it could limit the young patient's ability to discuss the options in depth, and give fully informed consent (Vindrola-Padros *et al.*, 2017).

Another review highlights that adolescents want health care professionals to discuss FP with them and not with their parents and would like to have the choice on who should be in the consultation(Quinn *et al.*, 2011a).

The FP team should have the ability to perform FP treatments on adolescent patients. It is important to register specific referral pathway, in which it may be necessary to refer to another clinic. It may be relevant to include a paediatrician in the team.

Conclusion

In addition to the general population, some specific recommendations can be made for adolescents:

- Adolescents should be given the option to have a consultation without their parents.
- The FP team should be aware of differences in legislation regarding informed consent (whether to be signed by adolescent or parents).
- In referral pathways, specific options should be present for adolescent patients.

Key organisational features for establishing a FP program

As discussed above, a high-quality FP program requires a multidisciplinary approach and should aim to overcome barriers to access FP care and interventions for different types of patients, while being in line with the legal context of the country. A checklist summarizes the requirements of a high-quality FP program (see Checklist 1).

Substantial differences will occur between different countries, reflecting variation in the organization of clinical care and the legal basis for provision of reproductive medicine and fertility preservation. Thus, the list of requirements outlined in Checklist 1 should not be considered comprehensive or exhaustive. The current list is partly based on published checklists (Andersen *et al.*, 2018), but was adapted by the guideline group to be applicable for the patient groups covered in the current guideline. The checklist is offered as an aid to establishing a FP program, or to evaluate an existing FP program against best practice.

The need for data collection

In order to increase the quality of care of FP, data collection by national and international registries on the short and long-term outcome of FP interventions are strongly recommended.

Since 2018 (data collection for 2015), ESHRE started collecting data through the ESHRE IVF monitoring scheme (EIM) in an optional module. Data are collected on the number of interventions, the reason for FP (being medical- or non-medical), and on the outcomes (number stored and number used) for 3 indications (in females), i.e. prepubertal ovarian tissue collection and cryopreservation, post pubertal ovarian tissue collection and cryopreservation, and oocyte cryopreservation. FP centres should contribute to national and international registries to optimize the quality and comprehensiveness of the data collected.

[24]

Checklist 1 Checklist for a high-quality FP program

An FP program should fulfil the following requirements:

- ✓ The legal framework of the country should be considered with regards to i) administrative/legal facilities agreement, ii) authorization and accreditation when imposed by local/national regulatory authorities; iii) ethical approval for aspects that are considered research.
- ✓ Referral pathways need to be established and require continuous maintenance.
- ✓ The following material and methods should be available:
  - Appropriate equipment
  - Qualified/authorized personnel (training programs)
  - Standard operating procedures (SOP):
    - Manipulation procedures
    - Cryopreservation procedures
    - Transport conditions
    - Media conditions
  - Certified and/or registered media/supplements and equipment used as per local legislation
- ✓ Administrative forms related to patients' assessment should be available, including:
  - Oncologists/other medical specialists written approval for FP, where appropriate
  - Report containing diagnosis and status of the disease and medical treatment proposed
  - Assessment and recording of patient's medical history, including assessment of specific factors relevant to FP e.g. risk of thrombosis/infection, previous treatment that may impact ovarian reserve/response to ovarian stimulation
  - Assessment of patient's serology (obligatory as part of regulatory rules in some countries)
- ✓ Multidisciplinary staff should officially participate in decision-making
- ✓ Written informed patients consent forms should be available outlining the following:
  - the risks/benefits of the procedure/intervention to be applied to recipient and to their gametes/tissue; it is suggested to use the EuroGTPII tool (http://www.goodtissuepractices.eu/)
  - the known or unknown outcomes
  - any applicable age limits or other criteria for using cryopreserved oocytes/embryos or ovarian tissue
  - choices regarding the destiny of the material in case of non-use within centre's determined period of time, for instance disposal, or donation for research
  - acknowledging centres policy for long-term storage, including time limitations and costs.

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A2. Legal aspects and availability

Data on whether fertility preservation is allowed in European countries, for which indications and under which conditions, were collected in an online survey. The details of the survey methodology are summarized in Annex 6 .

Data were collected from 30 countries (see table Table 1 to Table 4).

In general, oocyte cryopreservation for FP is allowed in all countries for which data were collected. Embryo cryopreservation for FP is also allowed in all countries except for Italy. For embryo cryopreservation, no data were available for Finland and Germany. Ovarian tissue cryopreservation for FP is allowed in 30 countries.

Cancer patients

Cancer patients requiring fertility preservation have the option of oocyte cryopreservation and embryo cryopreservation in all countries where these techniques are applied as part of fertility treatment (e.g. embryo cryopreservation is not allowed in Italy and Portugal) (see Table 1). Ovarian tissue cryopreservation for fertility preservation in cancer patients is allowed in all 28 countries for which data were available, although in 2 countries it is allowed only in a research context, and in 2 other countries it is allowed but not implemented.

In 15 countries (50,0%), there is no (or only very limited) coverage of costs for FP interventions. In 10 countries (33.3%), all available FP procedures are provided without costs to the patients, while in 5 countries (16,6%) at least one FP option is provided to patients without costs.

With regards to the conditions under which FP interventions are allowed or reimbursed, these are mostly related to patient characteristics (disease, prognosis, age) or limits on the number of treatments (number of cycles, first child). In Turkey, embryo cryopreservationis restricted to legally married couples.

Patients with benign diseases

Fertility preservation options for patients with benign diseases are similar to FP options for cancer patients in most countries, except for 2 countries, Czech Republic and Norway, where all 3 techniques are available for cancer patients (without and with cost coverage, respectively), but applying these techniques for patients with benign diseases is not allowed (see Table 2). Four other countries (Austria, Bulgaria, Ireland, Italy), allow the same treatments, but the coverage of costs seems to be more restricted in patients with benign diseases. Overall, there are 16 countries (53,3%) where there is no (or only very limited) coverage of costs, 4 countries (13,3%) where at least one FP option is provided to patients without costs, and 8 countries (26,6%) where all available FP procedures are provided without costs to patients with benign diseases, and 2 countries (as mentioned before) where FP service is not available. General conditions for applying FP in patients with benign diseases include scheduled gonadotoxic treatments, predictable impact on fertility, or (risk of) decreased ovarian reserve. In addition, restrictions on patient characteristics, number of treatments and specific restrictions on embryo cryopreservation exist and seem to be in line with those for cancer patients.

Transgender men

In contrast with cancer patients and patients with benign diseases, FP in transgender men is less well covered by local legislation, with 5 countries reporting a lack of regulation for FP in transgender men. In some of these countries, interventions are performed, although not covered by legislation, while in others interventions are considered not to be allowed (see Table 3). Oocyte cryopreservation for transgender men is allowed (or allowed under conditions) in 21 (70,0%) of 30

countries, embryo cryopreservation (often not preferred in transgender patients) is allowed in 14 countries (46,6%).

Several comments were made by the respondents on the restrictions with regard to the use of stored gametes or embryos after gender reassignment. In some countries, like Norway, FP techniques are not applied for transgender patients based on the requirement of using a surrogate, which is not legal in Norway.

Other countries reported specific requirements for the use of stored reproductive cells, although this was not a specific question. In Austria, reproductive cells should be donated to the partner. In Switzerland, preservation is allowed but the oocytes can only be used if the sex is not changed in the ID. In Croatia, use of the stored cells requires approval from the Ethical committee, which is also necessary in Belgium where additionally a medical and psychosocial screening is performed.

Financial support for transgender patients seems limited, with only 6 countries reporting provision of oocyte cryopreservation free-of-charge to this patient population.

FP for non-medical reasons

Elective oocyte cryopreservation is allowed in 21 (70.0%) of 30 countries (see Table 4). Of these countries, 3 reported that it is not regulated, and 2 reported it is only performed in the private setting (not in the public sector).

Conclusion

FP is available in most but not all European countries, thus specialists should be aware of their national legislative situation.

This generally supportive legislative environment applies to patients with cancer and benign diseases, and mostly to transgender men.

Provision of financial support is less widespread. This may reflect the rapidly developing nature of some FP procedures, and the ongoing change in their status from experimental towards being part of established care.

Table 1 Fertility Preservation options for cancer patients (per country) and information on the costs for patients

	Oocyte cryopreservation		Embryo cryopreservation		Ovarian tissue cryopreservation	
	Allowed?	Provided without costs for patients	Allowed?	Provided without costs for patients	Allowed?	Provided without costs for patients
Austria	V	No	V	Yes	V	Yes
Belgium	(V)[1]	Reimbursement under conditions[1,2]	(V)[1]	Reimbursement under conditions[1,2]	V	Reimbursement under conditions[1,2]
Bulgaria	V	Reimbursement under conditions	V	Reimbursement under conditions	V - RESEARCH	No
Croatia	(V)[1,3]	Yes	V	Reimbursement under conditions[1]	not implemented	
Cyprus	V	No	V	No	V	No
Czech Republic	V	No	V	No	V	No
Denmark	V	Yes	V	Yes	V	Yes
Finland	V	No				
France	V	Yes	V	Yes	(V)[3]	Yes
Georgia	V	No	V	No	V	No
Germany	V	No				
Hungary	(V)[5]	No[8]	(V)[5]	No[8]	V - RESEARCH	Yes (clinical trial)
Ireland	V	Yes	V	Yes	V no service available	No
Italy	V	Yes	X	No	V	Yes
Lithuania	V	No	V	No	V	No, partly
Montenegro	V	No	V	No	V	No
Netherlands	V	Yes	V	Yes	V	Yes
Norway	V	Yes	V	Yes	V	Yes
Poland	V	No	V	No	V	No
Portugal	V	Yes	X	No	V	Yes
Romania	V	No	V	No	V	No
Russian Federation	(V)	No	V	Reimbursement under conditions	V	No
Serbia	V	Yes	V	Yes	(V)	under conditions
Slovenia	V	Reimbursement under conditions[1]	V	Reimbursement under conditions[1,2]	V	Yes
Spain	V	Yes	V	Yes	V	Yes
Sweden	V	Yes	V	Yes	V	Yes
Switzerland	V	No[9]	V	No	V	No
Turkey	V	No[6]	(V)[7]	No[6]	V	No[6]
Ukraine	V	No	V	No	V	No
United Kingdom	V	Yes – but variable provision	V	Yes – but variable provision	V	Yes – but variable provision

V Allowed
(V) Allowed under conditions (specified where available)
X Not allowed
V - RESEARCH Allowed as experimental procedure or in a research context
1 Conditions related to age
2 Conditions related to number of treatments
3 Conditions related to prognosis / depending on indication explained in multidisciplinary consultation meeting
4 Conditions related to first child
5 Conditions related to the type of disease
6 depending on the IVF centre
7 Only for legally married couples
8 Storage fees
9 costs for medication are covered (by companies)

Table 2 Fertility Preservation options for patients with benign diseases (per country) and information on the costs for patients

	Oocyte cryopreservation		Embryo cryopreservation		Ovarian tissue cryopreservation	
	Allowed?	Provided without costs for patients	Allowed?	Provided without costs for patients	Allowed?	Provided without costs for patients
Austria	(V)[1]	No	(V)[6]	Yes	(V)[1,6]	No
Belgium	(V)	Reimbursement under conditions[3]	(V)[3]	Reimbursement under conditions [3,10]	V (not regulated)	Reimbursement under conditions
Bulgaria	V	No	V	No	V - RESEARCH	No
Croatia	(V)[3]	Yes	V	Yes	Not implemented	
Cyprus	V	No	V	No	V	No
Czech Republic	X	No	X	No	X	No
Denmark	V	Yes	V	Yes	V	Yes
Finland	(V) (not performed)	No				
France	(V)[4]	Yes	V	Yes	(V)[2,3]	Yes
Georgia	V	No	V	No	V	No
Germany	V	No				
Hungary	(V)[4]	No[9]	(V)[4,6]	No[9]	V - RESEARCH	Reimbursement under conditions
Ireland	V	No, partial reimbursement	V	No, partial reimbursement	V	No
Italy	V	??	X	No	V	Reimbursement under conditions
Lithuania	(V)[5]	No	(V)[5]	No	(V)[5]	No
Montenegro	(V)[2]	Reimbursement under conditions[3]	V	No	V	No
Netherlands	V	Yes	V	Yes	V	Yes
Norway	X	No	X	No	X	No
Poland	V	No	V	No	V	No
Portugal	V	Yes	X	No	(V)	Yes
Romania	V	No	V	No	V	No
Russian Federation	V	No	V	Reimbursement under conditions	V	No
Serbia	(V)[2]	Yes	(V)[4]	Reimbursement under conditions[4]	Not implemented	Reimbursement under conditions
Slovenia	V	Reimbursement under conditions[3]	V	Reimbursement under conditions[3,10]	V	Yes
Spain	V	Reimbursement under conditions	V	Yes	V	Yes
Sweden	V	Yes	V	Yes	V	Yes
Switzerland	V	No	V	No	V	No
Turkey	(V)[6]	No	(V)[8]	No[7]	(V)[6]	No[7]
Ukraine	V	No	V	No	V	No
United Kingdom	V	Yes – but variable provision	V	Yes – but variable provision	V	Yes – but variable provision

V	Allowed
(V)	Allowed under conditions (specified where available)
X	Not allowed
V - RESEARCH	as experimental procedure or in a research context
1	Endometrioma and POI
2	Restrictions on indications
3	Restrictions on age
4	Predictable impairment of fertility
5	Rare diseases
6	Decreased ovarian reserve or risk factors for decreased ovarian reserve
7	Depending on the IVF centre
8	Only for legally married couples
9	Storage fees
10	Conditions related to number of treatments

Table 3 Fertility Preservation options for transgender men (per country) and information on the costs for patients

	Oocyte cryopreservation		Embryo cryopreservation		Ovarian tissue cryopreservation	
	Allowed?	Provided without costs for patients	Allowed?	Provided without costs for patients	Allowed?	Provided without costs for patients
Austria	(V)	No	X	No	X	No
Belgium	(V)[1]	No	(V)[1]		V[3] (not regulated)	No
Bulgaria	(V)	No	(V)	No	V - RESEARCH	No
Croatia	(V)	No	X	No	Not implemented	
Cyprus	V	No	V	No	V	No
Czech Republic	X	No	X	No	X	No
Denmark	V	Yes	V	Yes	V	Yes
Finland	(V)[2]	No				
France	V	Yes	X	No	X	No
Georgia	X	No	X	No	X	No
Germany	V	No				
Hungary	X (not regulated)	No	X (not regulated)	No	X (not regulated)	No
Ireland	V	No	V	No	V	No
Italy	V	No	X	No	V	No
Lithuania	X	No	X	No	X	No
Montenegro	V (not regulated)	No	V (not regulated)	No	V (not regulated)	No
Netherlands	V	Yes	V	Yes	V	Yes
Norway	X	No	X	No	X	No
Poland	X	No	X	No	X	No
Portugal	V	Yes	X	No	X (not regulated)	No
Romania	(V) (not regulated)	No	(V) (not regulated)	No	(V) (not regulated)	No
Russian Federation	(V)	No	(V)	No	X	No
Serbia	X (not regulated)	No	X (not regulated)	No	X	No
Slovenia	X	No	X	No	X	Under conditions
Spain	V	Yes	V (in private system)	No	V (in private system)	No
Sweden	V	Yes	(V)	Under conditions	V	No
Switzerland	(V)	No	(V)	No	(V)	No
Turkey	X	No	X	No	X	
Ukraine	(V) (not regulated)	No	(V) (not regulated)	No	(V) (not regulated)	No
United Kingdom	V	Yes –variable/ limited provision	(V) (not performed)	Yes –variable/ limited provision	(V) (not performed)	Variable provision

V	Allowed
(V)	Allowed under conditions (specified where available)
X	Not allowed
V - RESEARCH	as experimental procedure or in a research context
1	Age restrictions
2	In Finland to be sterile before sex-change, law is changing. So far not done for transgenders in public side. Private clinics do preserve oocyte for transgenders.
3	Oocyte preservation is presented as a first option

76 Table 4 Options for non-medical fertility preservation (per country) and information on the costs
77 for patients

	Oocyte cryopreservation	
	Allowed?	Provided without costs for patients
Austria	X	No
Belgium	(V)[1]	No
Bulgaria	V	No
Croatia	V (not regulated)	No
Cyprus	V	No
Czech Republic	X	No
Denmark	V	No
Finland	V[2]	No
France	X[3]	No
Georgia	V	No
Germany	V	No
Hungary	X	No
Ireland	V	No
Italy	V	No
Lithuania	X	No
Montenegro	(V)[1] (not regulated)	No
Netherlands	V	No
Norway	X	No
Poland	X	No
Portugal	(V)[2]	No
Romania	V	No
Russian Federation	V	No
Serbia	X	No
Slovenia	X	No
Spain	V	No
Sweden	V	No
Switzerland	V	No
Turkey	(V)[4]	No
Ukraine	V (not regulated)	No
United Kingdom	V	No

V Allowed
(V) Allowed under conditions (specified where available)
X Not allowed
1 Age restrictions
2 Performed in private clinics only
3 The only non-medical condition for oocyte cryopreservation is as follows: An oocyte donor can preserve oocytes for herself if she has more than 5 oocytes retrieved:
4 Decreased ovarian reserve or risk factors for decreased ovarian reserve is the main condition to be fulfilled with no age upper or lower limit.

78

79

A3. Storage of reproductive material

Narrative Question: How long should reproductive material (oocytes, embryos, ovarian tissue) be stored?

Data on whether fertility preservation is allowed in European countries, for which indications and under which conditions were collected in an online survey. The details of the survey methodology are summarized in Annex 6.

Through the survey, data were collected from 29 countries (see Table 5).

With data for 29 countries, 11 (37.9%) reported that both storage and use of stored oocytes were limited, 4 (13.8%) reported that storage was limited (without limits for use of stored oocytes), and 6 (20.6%) reported that only the use was limited, but not the storage. With regards to storage, a duration of 5 or 10 years is most often reported, and this is mostly extendable. The age limit for use of the oocytes is reported to be ranging from 42 to 55 years. Eight countries reported that there were no limits for duration of storage of oocytes, nor for the use of the stored gametes.

Similar results were found for embryo cryopreservation: 10 (34.5%) of 29 countries reported limits for both duration of storage and age of use, 6 (20.6%) reported that storage duration is limited, 4 (13.8%) reported use of stored embryos was limited, 6 (20.6%) reported no limits.

Oocyte and embryo storage limitations were similar in most countries, except for Poland, Serbia and Sweden, which have limits for storage of embryos, but not for oocytes.

Storage of ovarian tissue is less well defined; 6 (23.0%) of 26 countries reported limits for storage, mostly 10 years and extendable. For the use of stored ovarian tissue, 10 (37.0%) of 27 countries reported that this was not regulated, or not included in the legislation, 7 (25.9%) countries stated that there was no limit, 10 (37.0%) countries apply an age limit between 40 and 50 years.

Conclusion

Regulations regarding the duration of storage of reproductive materials are very variable across Europe. Some contries also have different storage regulations for different materials.

While a duration of storage is often applied, this may be supplemented by an upper age limit for use.

Given the young age at which FP may occur, the often short allowable duration of storage (5-10 years in many countries) is inappropriate, and legislation should focus more on a maximum age of use.

106 Table 5 Duration of storage and age limit for use of stored material (oocytes, embryos and
107 ovarian tissue) in different countries.

	OOCYTES			EMBRYOS			OVARIAN TISSUE		
	Duration of storage		Age limit for use of stored material	Duration of storage		Age limit for use of stored material	Duration of storage		Age limit for use of stored material
Austria	Limited	lifetime	No limit	Limited	10 years	No limit	No limit		No limit
Belgium	Limited	10 years (extendable)	< 48 years	Limited	5 years (extendable)	< 48 years	Limited	10 years (extendable)	No legislation - no limit
Bulgaria	Limited	5 years (extendable)	No limit	Limited	5 years (extendable)	No limit	No limit		Not defined
Croatia	Limited	5 -10 years (extendable but paid by patient)	42 years of age	No limit		No limit			Still not being implemented
Cyprus	Limited	10 years (extendable)	50 years	Limited	10 years (extendable)	50 years	Limited	10 years (extendable)	50 years
Czech Republic	No limit		No limit	No limit		No limit	No limit		No limit
Denmark	Limited	5 years	35 years	Limited	5 years	45 years			
Finland	Limited	Defined by clinic	Defined by clinic - 40 to 50 years.						
France	No limit		Current practice is 40 - 45 years	No limit		Current practice is 43 to 45 years, or 51 years	No limit		Current practice is 42-43 years
Georgia	No limit		Not regulated	No limit		Not regulated	No limit		Not regulated
Germany									
Hungary	Limited	10 years	< 50 years	Limited	10 years	< 50 years	No limit		Not regulated (< 50 years)
Ireland	No limit		No limit	No limit		No limit	No limit		No limit
Italy	No limit		50 years	No limit		50 years	No limit		50 years
Lithuania	No limit		No limit	No limit		No limit	No limit		No limit
Montenegro	Limited	Defined by clinic, current practice is 2 times 3-5 years	Defined by clinic - 48 years	Limited	Current practice is 3+3 years	Not regulated, current practice is 45-47 years	No limit		Not regulated
Netherlands	No limit		49 years	No limit		49 years	No limit		49 years
Norway	Limited	Not defined, extending natural fertility is not allowed	Defined by clinic - 45 years	Limited	5 years	Current practice is age at OPU + 5 years.	No limit		No limit, current practice is 45 years.
Poland	No limit		Not defined	Limited	20 years1	Not defined	No limit		Not defined
Portugal	Limited	5 years (extendable)	< 50 years	Limited	3 years (extendable for 3 years)	< 50 years	Limited	5 years (extendable)	< 50 years
Romania	No limit		Not regulated, current practice is 50 years	No limit		Not regulated, current practice is 50 years	No limit		Not specified

[1] After which it is transferred to the tissue bank for obligatory anonymous donation

[34]

	OOCYTES			EMBRYOS			OVARIAN TISSUE		
	Duration of storage		Age limit for use of stored material	Duration of storage		Age limit for use of stored material	Duration of storage		Age limit for use of stored material
Russian Federation	No limit		No limit	No limit		No limit	No limit		No limit
Serbia	No limit		Not specified in legislation	Limited	5 years (extendable to 10 years)	Not specified in legislation	No limit		Not specified in legislation
Slovenia	Limited	10 years	Women of reproductive age.	Limited	10 years	Women of reproductive age.	Limited	10 years	Women of reproductive age.
Spain	No limit		40 years			40 years	No limit		40 years
Sweden	No limit		45–50 years	Limited	10 years	45–50 years	No limit		45–50 years
Switzerland	Limited	10 years, unless stored for medical reasons.	No limit	Limited	10 years	No limit	Limited	10 years, unless stored for medical reasons.	No limit
Turkey	Limited	5 + 5 years (extendable)	No limit	Limited	10 years	No limit	Limited	10 years	Not regulated
Ukraine	No limit		Defined by infertility specialist	No limit		No limit	No limit		Defined by infertility specialist
United Kingdom	Limited	10 years (extendable under conditions)	No limit, current practice is 50 – 55 years	Limited	10 years (extendable)	No limit, current practice is 50 – 55 years	No limit		No limit

PART B: Patient information

B1. Information needs and provision

Receiving information about the effect of cancer treatment or other treatments in future fertility is essential in supporting decision-making to undergo fertility preservation. Nevertheless, there is still lack of information provision in patients facing infertility risk (Baram *et al.*, 2019, Patel *et al.*, 2020). In a recent study about provision of information in men and women facing cancer treatments, only 74.5% recalled having this discussion with their physician, and about 17% of them had the discussion after starting chemotherapy (Patel *et al.*, 2020). Of those patients who did not recall having FP discussion, 83.3% would have liked to have it. Of the patients who did not pursue fertility treatments, 41.6% reported that they were not aware of any options. Therefore, even when patients are informed about the risk of infertility, it is clear that provision of information is not always well performed and very often patients are not informed about FP options (Logan *et al.*, 2019, Patel *et al.*, 2020).

Being informed about the possibility to undergo fertility preservation was associated with decreased decisional conflict in a sample of former cancer patients aged 18-45 years old (Muller *et al.*, 2017). Similarly, decreased knowledge was associated with increased decisional conflict about pursuing fertility preservation in women aged 28-40 years by the time of cancer diagnosis (Peate *et al.*, 2011). Contradicting findings were reported by a study by Kim et al, where all women had a prior fertility preservation consultation (Kim *et al.*, 2013). In this study the association between knowledge about FP and decisional conflict was non-significant which can suggest that it is not the degree of knowledge but not being informed about FP options that increases decisional conflict. Nevertheless, we cannot exclude a negative reaction to be informed about risk of infertility, as some patients might find this information difficult to handle (Crawshaw *et al.*, 2009).

Narrative Question: Which information needs to be provided to women at risk of infertility?

A systematic review by Peate el al. conducted in 2009 retrieved twenty studies evaluating fertility-related information needs, concerns and preferences of young women with breast cancer (Peate *et al.*, 2009). Three themes emerged regarding fertility related psychosocial needs and concerns: Needs regarding changes in menstrual cycle and potential infertility; attitudes and decisions regarding pregnancy (effects of pregnancy on cancer recurrence, breastfeeding and contraception); and fertility related information needs. Specifically, regarding fertility related information needs, some studies found that fertility issues affected their cancer treatment decision-making. More recently, another systematic review conducted by Goossens et al., in 2014, reviewed 27 papers assessing fertility information needs and receipt and provision of information (Goossens *et al.*, 2014). Twenty-one of these studies focused on the patient perspective, that is, which information do patients need. Main information needs were about the gonadotoxic impact of malignancy and of cancer treatments on fertility (even in situations where FP options were not available), pre-treatment fertility information and post-treatment reproductive life planning, fertility options, risk of infertility, amenorrhea and premature ovarian insufficiency.

In 2018, Silva and colleagues reported a literature research on patients' information needs concerning infertility risks and FP options (Silva *et al.*, 2018). Ten published articles were analysed, and several themes emerged, namely menstrual changes after cancer and cancer treatment, impact of cancer treatment in fertility and risk of infertility, infertility options, cryopreservation related issues, infertility treatments and pregnancy planning and pregnancy risks after cancer (see information needs list). Information needs can vary according to the phase of cancer diagnosis and treatment (Goossens *et al.*, 2014, Shen *et al.*, 2019).

The information needs regarding fertility preservation in transgender men has been scarcely addressed in the literature, but lack of awareness of FP options in transgender people has been documented in a recent systematic review (Baram *et al.*, 2019). Regarding fertility preservation for non-medical reasons, a study that surveyed 580 young women reported that 28% of them would like to receive education on their FP options and 36% responded they would like their gynaecologist to discuss FP options (Hickman *et al.*, 2018).

In a study examining decision regret in a sample of 201 women who underwent oocyte cryopreservation for non-medical reasons, 80% of the participants reported having had adequate information when deciding to undergo FP. The perception of having adequate information was associated with reduced risk of regret (Greenwood *et al.*, 2018).

In a study examining decision regret in a sample of 201 women who underwent oocyte cryopreservation for non-medical reasons, 80% of the participants reported having had adequate information when deciding to undergo FP. The perception of having adequate information was associated with reduced risk of regret (Greenwood *et al.*, 2018).

Recommendations

Clinicians should provide information to patients regarding 1) impact of cancer, other diseases and their treatments on reproductive function; 2) impact of cancer, other diseases and their treatment on fertility, 3) fertility preservation options; 4) cryopreservation related issues after FP, 5) infertility and fertility treatments; 6) pregnancy after cancer; and 7) other childbearing and parenting options.	**STRONG** ⊕⊕○○
Information provided should be specific to the patients' needs.	**GPP**
Age-specific information and counselling should be provided for adolescents and young adults.	**GPP**

Justification

The recommendation on information provision is based on (moderate quality) evidence in cancer patients showing the importance of receiving information about FP and which specific needs patients have (Peate et al., 2009, Goossens et al., 2014, Silva et al., 2018). There is no direct evidence on the information needs of patients at risk of infertility due to other medical situations, gender reassignment therapy or elective oocyte cryopreservation, but it seemed relevant to expand the recommendations to be also applicable to these patient groups (based on indirect evidence from cancer patients). Although the information can be stressful and difficult to handle by some patients, being informed about the possibility of FP is associated with better outcomes (the benefits seem to outweigh the harms). Patients highlight the importance of having material to support their decision-making.

PICO Question: How should information on fertility preservation options be provided to patients?

Decision-making regarding fertility preservation is stressful. Provision of information and fertility counselling are of great importance to allow for high-quality decision-making. There is great variability across studies regarding the proportion of patients receiving information about cancer related infertility (between 0% and 85%) and regarding the satisfaction with information received by

cancer patients undergoing fertility preservation, with percentage of patients evaluating the information received as sufficient ranging from 11% to 90% (Goossens *et al.*, 2014).

Transgender patients often felt that information, even when provided, was incomplete, which affected patients' satisfaction with decision-making (Chen *et al.*, 2019).

An evaluation of gynaecologists' and obstetricians' knowledge and practices regarding counselling and provision of information of women with childbearing plans and delaying pregnancy for social reasons showed that only 27.6% of participants counselled women about age related fertility decline, although 58.1% were asked about elective freezing by their patients (Fritz *et al.*, 2018).

Patient preferences

A systematic review on fertility related concerns in young women with breast cancer evaluated preferences for provision of fertility-related information (Peate *et al.*, 2009). One of the studies reported that the most preferred method for obtaining fertility related information was a consultation with a fertility specialist followed by a decision aid early in the treatment plan.

Another systematic review using a mixed methods approach retrieved 27 papers reporting fertility information needs and provision preferences, 21 of them focusing on the patients' perspective (Goossens *et al.*, 2014). Results highlighted that patients preferred to be informed during an individual consultation by a fertility specialist or by an oncologist, ideally about one week after cancer diagnosis, after recovering from the shock of the cancer diagnosis, and prior to cancer treatment. Similarly, Anazodo's systematic scoping identified that patients and patients' parents preferred to receive FP information by the time of the cancer diagnosis (Anazodo *et al.*, 2019).

Written information was considered as a supplement to oral information (Goossens *et al.*, 2014, Shen *et al.*, 2019). Studies have documented that patients valued the possibility of written information that they could take home and be able to read again, before or after receiving the information regarding FP (Garvelink *et al.*, 2015, Ehrbar *et al.*, 2016, Kelvin *et al.*, 2016, Vogt *et al.*, 2018) or a website with information available (Garvelink *et al.*, 2012, Muller *et al.*, 2017). A study by Tam et al. (2018) with cancer patients and their partners reported that 93% of female patients found the use of brochures useful (Tam *et al.*, 2018). Participants preferred to receive FP information verbally (73%), in writing (66%) or in a website (57%). Videos (21%) and education (11%) were the least preferred methods. These results are in line with preferences reported by Speller et al. (2019), who reported that 88% of study participants (patients and health care providers) preferred paper and/or online resources over other formats (audio guided booklet or videos) (Speller *et al.*, 2019c). Borgmann-Staudt and colleagues developed an educational intervention study with cancer patients and their parents (Borgmann-Staudt *et al.*, 2019). In this study, a control group received standard patient education and the intervention group received an additional information flyer at initial diagnosis. Results showed an increase in knowledge and in feelings of empowerment in the intervention group, and effects were higher in female patients, older patients and the highly educated.

A narrative review by Jones and colleagues examined the factors that hindered the decision making of women with cancer contemplating FP (Jones *et al.*, 2017a). External and internal factors were found to affect decision-making, underlining the importance of considering patients subjective factors. Indeed, this review highlighted that the decision-making to pursue FP was affected by fears related to the FP treatment, which evoked the dilemma of which treatment should be prioritized. In line with this, in the qualitative study by Srikanthan et al. (2019), patients reported the importance of having their preferences and personal situations addressed (Srikanthan *et al.*, 2019). Therefore, tools that support FP decision-making should not only inform patients about their FP options but also take into consideration their specific values and preferences.

Transgender patients' preferences about receiving FP information has received less attention. A mixed methods systematic review included 27 studies on fertility care for transgender men evaluating satisfaction with information provided and preferences regarding methods of information provision (Johnson *et al.*, 2016). Results showed that patients expected to receive information in a consultation and written information was considered supplementary. Although

some patients considered written information useful, especially to revisit the information when needed, some patients reported that written information was not concise, and they felt overwhelmed by information.

Decision aids to support patients' decision-making

Decision aids (DAs) are tools or interventions based on education materials that aim to provide information to patients to support their treatment-related decisions. These materials, like other information tools (e.g. informative sheet), provide information about each of the available options and about potential harms and benefits. DAs differ from informative sheet by explicitly eliciting patient's preferences and/or values regarding each option and asking the patients about their final choice or preferred choice, therefore improving congruence between decisions and personal values. Decision aids can be used by clinicians and by patients and either in preparation, during or after the clinical consultation. Decision aids can be printed, or web based, but there are no studies comparing the effectiveness and patient's satisfaction between these types of DAs in FP decision.

In the last years several DAs to support FP decision in women of reproductive age with cancer have been developed. Speller et al. (2019) examined the quality of 31 DAs and other support resource materials (Speller *et al.*, 2019b). Specifically, the quality of DAs was evaluated using the International Patient Decision Aid Standard Collaboration Checklist, with several of the DAs included in the review (Peate *et al.*, 2011, Peate *et al.*, 2012, Garvelink *et al.*, 2013) rated as high quality.

The effectiveness of the use of DAs in FP decision was examined, evaluating improvement in knowledge, decisional conflict, satisfaction and acceptability and regret (Wang *et al.*, 2019). Decision aids proved to be effective in improving knowledge in three studies and, in one sample, knowledge was retained for 6 months. Specifically, the use of DA in addition to standard care or fertility counselling was associated with increased knowledge. Decisional conflict decreased after the use of a DA, as reported by two studies in female cancer patients. However, when compared to the use of a brochure only or counselling only, there were no differences between these two interventions or the use of the DA. The authors concluded that existing studies did not provide clear evidence on the benefit of DAs for decreasing decisional conflict (Wang *et al.*, 2019). Satisfaction with the use of DAs and acceptability were also assessed in the review, and results are indicative of positive assessment after the use of DAs. Patients and clinicians reported that DAs were easy to read, well organized and contained relevant information and more than 88% would recommend their use (Wang *et al.*, 2019). Nevertheless, some negative feeling after the use of the DAs were also reported.

Finally, decisional regret was also evaluated in this review (Wang *et al.*, 2019). There were no differences in regret at the time of the decision or 6 months after, but at 12 months after the decision, regret was significantly lower in the group who used the DA when compared with standard care.

Similar results were found in a study of the effect of the use of an online DA (FERTIONCO) in addition to standard counselling by a FP specialist (Ehrbar *et al.*, 2019). Women who used the DA reported lower decisional conflict after counselling and one month later. Additionally, more women had decided for or against FP in the group using the DA compared to the control group at the first assessment (i.e. immediately after consultation or use of the DA). Satisfaction with the use of the DA was positive and more than 80% of the participants would recommend the use of the DA.

Recommendation

It is recommended to provide decision aids to patients who are considering FP.	**STRONG** ⊕⊕◯◯

Justification

Making general conclusions on the efficacy of DAs is troubled by the limited studies that examined the efficacy of the DAs, the quality of the studies (although summarized in systematic reviews) and by each assessing different interventions and outcomes. Overall, available evidence was felt to show some

benefit of DAs, while the risks are minimal and, when existing, limited to an increase in negative emotions reported in some patients (Wang et al., 2019). Patients report the need of a tool that includes more information on FP options and helps them in the decision-making process. Decision aids can be long documents, which can diminish its use, but overall, providing patients with DAs is considered acceptable and feasible.

Examples of published DAs are listed in Table 6.

Table 6 Decision aids that are currently available to patients and/or which have been shown to be effective in supporting the FP decision making

Decision aid /Reference	Online version	Language	Tool modality	Components included	Effectiveness
Decision aids with effectiveness studies published					
Fertility related choices (Peate *et al.*, 2011, Peate *et al.*, 2012)	Available here	English	Booklet available online	• Information about cancer, fertility and FP options; • Value clarification exercises • Includes a balance sheet to weight and compare options	• Decrease in decisional conflict • Decrease in decisional regret • No change in anxiety or depression symptoms • Increase in knowledge • Satisfaction with information received
(Garvelink *et al.*, 2013, Garvelink *et al.*, 2017)	Not available	Dutch	Online tool	• Information about cancer, fertility and FP options; • Value clarification exercises	• Increase in knowledge • Slightly higher Decisional conflict compared to use of brochures
Fertionco (Ehrbar *et al.*, 2018, Ehrbar *et al.*, 2019)	Available here	German French	Online tool	• Information about cancer, fertility and FP options; • Value clarification exercises • Includes a weighting and deciding tool that allows for a sum of arguments in favour and against each option.	• Lower decisional conflict • Less time to take the decision • Higher Satisfaction
Decision aids without effectiveness studies published (ongoing studies)					
Cancer, Fertility & Me (Jones *et al.*, 2017b)	Available here	English	Website and printable version	• Information about cancer and FP options • Decision-making exercises	Effectiveness results not published yet
Pathways patient decision aid website (Woodard *et al.*, 2018)	Not available	English	Online tool	• Information about cancer, fertility and FP options; • Value clarification exercises	Effectiveness results not published yet Acceptability studies
The "Begin Exploring Fertility Options, Risks and Expectations" (BEFORE) (Speller *et al.*, 2019a)	Available here	English	Website and printable version	• Information about cancer, fertility and FP options; • Fertility options exercise to help patients making to decision	Effectiveness results not published yet
DA for parents with Children and Adolescents with cancer (Allingham *et al.*, 2018)	Not available	English	Online tool	• Information about cancer, fertility and FP options; • Information on how parents can talk with their children about fertility and fertility preservation • Value clarification exercises	Acceptability studies

Research recommendation

Studies are needed comparing the effectiveness and patients' satisfaction with written compared to online DAs. The relevance of the DAs in supporting patients' decision making and reducing emotional distress at the time of the decision should be further clarified.

Tools to support clinicians in providing FP information to patients

Several studies also have documented that healthcare professionals have difficulties in discussing cancer-related infertility risks and FP options. In a study by Kemertzis et al., 66% of healthcare providers (nurses, clinicians and allied health professionals) reported dissatisfaction with existing FP system and 59.6% were not confident in providing up-to-date FP information (Kemertzis *et al.*, 2018). In the same study, 34.5% of respondents reported providing (often or always) verbal (oral) information and 14% reported providing written information. A mixed methods systematic review of healthcare professionals' views on discussing FP with children, adolescents and young cancer patients (aged 0-24) retrieved 16 papers reporting 14 studies (Vindrola-Padros *et al.*, 2017). In this review, seven studies reported that healthcare professional did not have educational material to support FP discussions and in two of these studies professionals reported to be more likely to discuss FP options if they had educational materials. Anazodo's systematic scoping review on models of care examined 30 papers addressing cancer clinician knowledge and training and 20 papers addressing knowledge and training in non-cancer clinician. Studies reviewed highlighted that health care professionals wanted more educational materials and education to provide fertility care (Anazodo *et al.*, 2019). Therefore, it seems important to provide patients and clinicians with materials to support FP discussions.

One study evaluated the effect of the use of a checklist ("fertility toolkit") for healthcare providers who discuss FP options with children, adolescents and young adult patients and their parents (Kemertzis *et al.*, 2018). A survey was used to assess implementation and impact of the toolkit three time points: baseline, after use and 2 years after the toolkit introduction. After the use of the toolkit, healthcare providers reported a significant improvement in confidence levels regarding the provision of information, although satisfaction with FP discussion was not significantly increased. The healthcare providers reported a significant improvement in the provision of verbal and written information. This toolkit was further developed and revised into a clinician decision support system (CDSS), i.e. a computer application design to aid clinicians in supporting decisions in patient care (Hand *et al.*, 2018). The authors examined the usability and acceptability of this CDSS in a sample of 39 clinical staff working in an oncofertility care unit. In this study, more than 60% agreed that this CDSS would enable adherence to consistent clinical pathways, policy and standards of care and would improve clinician consistency in provision of information and patient and family decision making. A total of 96,2% reported willingness to lead fertility discussions using the CDSS, indicating high levels of acceptance of the tool. No studies on the effectiveness on patients' outcomes of using these tools have been published.

One survey study evaluated clinicians' preferences working with transgender people regarding the use of decision aids or a provider assessment tool, with most clinicians (i.e. 67%) reporting a preference for the use of a decision aid (Johnson *et al.*, 2016).

Recommendations

Healthcare professionals may consider the use of a checklist for a better provision of information to patients.	**WEAK** ⊕○○○

Justification

Overall there is very little evidence on the use of tools for clinicians to assist them in providing fertility-related information to patients. The study by Kemertzis provides indirect evidence for the current guideline as it reports on a paediatric oncology setting (Kemertzis et al., 2018). In addition, there are no data on patient's satisfaction or other outcomes after consultation with or without tools for clinicians

on fertility issues. However, given the evidence for fears of healthcare professionals to provide FP information and the patient's needs and preferences for information provision on fertility issues, and the lack of risks associated with it, healthcare professionals may consider the use of a checklist or a toolkit to improve the provision of information to patients (due to fears of HCP in providing info).

Based on the information needs reported for women undergoing FP due to a cancer diagnosis (Goossens *et al.*, 2014, Silva *et al.*, 2018), we have developed a list of information needs of women undergoing fertility preservation, to support clinicians in providing all relevant information (see Checklist 2).

247 **Checklist 2 Checklist for clinicians to cover the Information needs of patients undergoing fertility**
248 **preservation counselling**

Information needs	Cancer patients	Medical (non-cancer) patients	Trans-gender men	Women undergoing FP for non-medical reasons
1) Impact of disease/treatment on reproductive function				
Menstrual changes/Amenorrhoea	√	√	√	-
Premature ovarian insufficiency	√	√	√	-
Information about contraception	√	√	√	-
2) Impact of disease/treatment on fertility				
Effects of disease on fertility	√	√	-	-
Effects of treatments on fertility / risk of infertility	√	√	√	-
Effects of hormonal therapy on fertility	√	√	√	-
3) Fertility preservation options				
Effects of hormonal stimulation for FP on disease recurrence	√	√	√	-
Impact of age at the time of FP on success rates	√	√	√	√
Fertility preservation options				
- Established and experimental FP techniques	√	√	√	√
- Time requirements of each FP option	√	√	√	√
- Success rates of each FP technique	√	√	√	√
- Pregnancy rates after each FP option	√	√	√	√
- Risks of each FP technique	√	√	√	√
- Side effects of each FP technique	√	√	√	√
- Advantages of each FP technique	√	√	√	√
- Disadvantages of each FP technique	√	√	√	√
- Costs of each FP technique	√	√	√	√
Late FP options[1]	√	√	√	
Ethical issues associated with embryo cryopreservation	√	√	√	√
4) Cryopreservation and storage of cryopreserved material				
Maximum time for cryopreservation	√	√	√	√
Costs of cryopreservation	√	√	√	√
5) Infertility and fertility treatments				
Infertility and Medically assisted reproduction treatments	√	√	√	√
6) Pregnancy after cancer				
Risk of disease recurrence due to pregnancy	√		-	-
Risks/benefits of having children after cancer/other diseases	√	√	-	-
Effects of disease/treatments on future children (repeated?)	√	√	√	-
Obstetric risks	√	√	√	√
7) Childbearing/Parenting options				
Reproductive planning after disease/treatment/other situations	√	√	√	√
Other options to achieve pregnancy/parenting	√	√	√	√

249 [1] Including FP after completion of cancer treatment or other treatments for non-malignant diseases.
250 For transgender men, this implies FP options after the start of gender-affirming hormone therapy

B2. Support and counselling

Fertility-related concerns and fertility preservation treatment have a significant psychological impact in cancer patients. A study by Takeuchi et al. reported that 14% of participants felt fear and shock when facing the risk of infertility, and frequently endorsed the need for psychological support (Takeuchi *et al.*, 2019). Similarly, in a prospective mixed methods study with women recently diagnosed with breast cancer, 'psychosocial factors' were an emergent theme, with women referring fear as a dominant emotion related with the cancer and fertility related issues (Vogt *et al.*, 2018). A systematic review included 47 papers examining fertility related psychological distress in cancer patients, from diagnosis to survivorship and reported that patients presenting for FP at the time of cancer diagnosis and treatment had poorer mental health when compared with infertile patients regarding depression, anxiety and fertility related stress (Logan and Anazodo, 2019). Another study included in the review showed that 1/3 of female cancer patients undergoing ovarian stimulation reported impairing symptoms of anxiety and depression. A systematic scoping review retrieved 14 papers discussing patients' negative emotional impact of infertility after cancer; this showed that the threat of infertility was associated with psychological distress and that patients want to receive more support (Anazodo *et al.*, 2019). It should be noted some cancer patients may have specific needs. For example, La Rosa and colleagues highlighted that gynaecological patients may require special FP and psychological counselling due to the serious impact that gynaecological cancers and its treatment may have on their future sexuality and female identity (La Rosa *et al.*, 2019).

Because distress, anxiety and depression can affect decision making, some patients may benefit from psychological counselling in addition to fertility counselling. While fertility counselling refers to the provision of information regarding infertility risks and FP options and is usually provided by a clinician, psychological counselling is targeted at exploring reproductive concerns and promoting strategies to deal with the stress of the decision in the short and long term (Logan and Anazodo, 2019).

PICO Question: Is there a benefit of psychological support and counselling, and are there particular groups that would benefit from it?

Is there a benefit of psychological support and counselling?

Studies on the effect of psychosocial support and counselling in the decision making of patients undergoing fertility preservation procedure are scarce. A systematic review on oncofertility support needs of cancer patients retrieved 30 papers and categorized the needs as information, service, clinician-patient interactions, psychological, and family (Logan *et al.*, 2018). Regarding psychological support needs, one study documented that female patients expressed the desire for additional support, such as specialized psychological service or a post treatment internet group. Another study documented that the presence of a psychologist in a fertility preservation team was considered helpful, although no specific psychological intervention was performed (Logan *et al.*, 2018). A systematic scoping review retrieved 14 studies discussing needs for emotional support. Of these, two studies highlighted that patients reported that emotional support was important at all stages of treatment and that counselling was useful in different time points due to the complexity of FP decision making (Anazodo *et al.*, 2019).

Chiavari and colleagues evaluated the effect of a decision-making support tool (based on decision counselling) on decision-making, decisional conflict and anxiety in cancer patients facing fertility-related decisions (Chiavari *et al.*, 2015). This study differentiated between the provision of information, which was provided by the clinician, and decisional support, which was focused in personal aspects that could influence the decision and improve satisfaction with the decision. The Decision Counselling (DeCo) intervention was conducted by health professionals with training in counselling. Results showed a statistically significant increase in stage of decision-making (which

reflects patients' readiness to engage in decision-making and progress in decisions) and a reduction in decisional conflict after the intervention. Changes were observed in the subscale of feeling informed and uncertainty of decisional conflict.

There are no studies evaluating effect of psychological counselling in a long-term adjustment for cancer patients referred for FP.

Regarding FP for non-medical reasons, one study assessed decisional regret in a sample of 201 women undergoing oocyte cryopreservation (Greenwood *et al.*, 2018). Decisional regret was found to be associated with perceived adequacy of information when deciding to pursue oocyte cryopreservation and perceived adequacy of emotional support during treatment. Caution should be used regarding these findings, because the participants reported on their perception of information and support in routine care, which was not standardized or described in detail.

Recommendation

It is recommended that patients are offered psychological support and counselling when dealing with FP decisions, although the extent of the clinical benefit has not been studied.	**STRONG** ⊕○○○

Justification

The evidence on the effect of psychological support on FP patients is weak and indirect, as there are no specific intervention studies with a control group. Existing studies do not provided evidence for the effect of psychological support on psychological (depression, anxiety, quality of life, regret) and FP outcomes (e.g. use of material), either short or long term.

Patients consider psychological support helpful when dealing with FP decisions and in absence of harms with such intervention, the GDG decided to recommend that psychological support is offered.

Offering psychological support and counselling will depend on the availability of a psychologist/counsellor in the FP team, and this may impact on the feasibility of the recommendation.

Selection of patients for psychological support and counselling

There are no studies providing direct evidence on subgroups of FP patients that would specifically benefit from psychological support. However, some studies examined predictors of emotional distress in cancer patients and reproductive concerns in FP patients (Shah *et al.*, 2016, Logan *et al.*, 2019).

O'Hea and colleagues showed that history of psychological problems (e.g. previous diagnoses, past use of psychotropic medications, or history of counselling or psychotherapy) was related to psychological distress in cancer patients (O'Hea *et al.*, 2016). Additionally, some psychological processes and fertility- or cancer-related variables were also associated with psychological distress. Specifically the odds of being diagnosed with depressive symptoms was related to higher levels of avoidance coping (Lawson *et al.*, 2014). Similarly, the odds of being diagnosed with anxiety symptoms was related to poor insurance coverage, higher sexual concerns and avoidance coping strategies (Lawson *et al.*, 2014).

In a retrospective evaluation of reproductive concerns in 356 female cancer survivorsreproductive concerns were higher among women that were i) younger at diagnosis; ii) treated for leukemia; iii) treated with chemoradiation or bone marrow transplantation; iv) nulliparous; v) desiring future children at the time of diagnosis; vi) infertile after treatment; or vii) had a lower income (Shah *et al.*, 2016).

Independent of FP, transgender men were reported to have a higher risk of depression than gender-congruent people (Witcomb *et al.*, 2018).

Conclusion

The multidisciplinary FP team counselling FP patients should be aware that maladaptive psychological processes and past psychopathology are risk factors for psychological distress during FP decision. It is recommended that patients at risk are referred for psychological support when needed.

Clinicians should be aware of risk factors for psychological distress during FP (e.g. past psychopathology, current exacerbated concerns or distress regarding future fertility)

Recommendation

Clinicians may consider referring FP patients who present risk factors for psychological distress for psychological support and counselling.	**WEAK** ⊕○○○

Justification

The key question aimed to identify certain patient subgroups that could have a significant benefit of psychological support and counselling. In absence of any direct evidence, information on predictors for maladaptation was collected, hypothesizing that such predictors could help selecting patients that have more benefit from psychological support and counselling. Predictors for psychological distress include:

- *past psychopathology*
- *maladaptive psychological processes*
- *current exacerbated concerns*
- *distress regarding future fertility*

For specific considerations for women attempting oocyte cryopreservation for non-medical reasons see D4. Oocyte cryopreservation for non-medical reasons

Research recommendation

Studies should investigate the benefit of providing psychological counselling to women undergoing FP decision-making. It should also be investigated which patients would benefit the most from psychological support and counselling. There is a need for more studies examining risk factors for emotional distress in patients undergoing FP.

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PART C: Patient selection and pre-FP assessment

C1. Patient selection

NARRATIVE QUESTION: WHICH CRITERIA CAN BE USED TO SELECT PATIENTS FOR FERTILITY PRESERVATION?

Many, or indeed most, young women treated for cancer will retain their fertility and it is therefore important to attempt to identify the degree of risk to allow informed patient decision-making, and to focus fertility preservation activities on those who are particularly at risk of loss of fertility. The factors that are relevant to determining this risk include the age at which the woman is treated, with increasing age associated with increasing risk; the treatment administered, reflecting the diagnosis and staging; and potentially individual factors within the patient that determine individual susceptibility, such as her ovarian reserve. The importance of some specific treatment modalities is well established with alkylating agent chemotherapy and radiotherapy to a field that includes the ovary being of particular dose-dependent high risk. Radiotherapy to a field that includes the uterus is also important in relation to the ability to carry successfully a pregnancy to term.

While fertility preservation (FP) is generally considered and conducted in two parts, i.e. the cryopreservation of gametes or gonadal tissue initially, with later attempts to achieve a pregnancy, the latter should always be considered at the time of the former. Thus, implicit in the patient evaluation at initial presentation is consideration of the potential for a successful pregnancy and what risks the patient's health, and the proposed treatment on for example, cardiac function, must be considered.

Checklist 3 provides a proposed structure for patients' assessment and selection that can be used in this regard.

Checklist 3 Proposed structure for patients' assessment and selection that can be used in this regard (adapted from (Wallace *et al.*, 2012))

Intrinsic factors

- ✓ Health status of patient
  - Surgical/anaesthetic risk, including thrombosis and infection
  - Malignant contamination of the ovary
- ✓ The need to obtain fully informed consent (patient/parent)
- ✓ Age (upper and lower limits for safety and efficacy)
- ✓ Assessment of ovarian reserve

Extrinsic factors

- ✓ Nature of predicted treatment
  - High/medium/low/uncertain risk of POI/infertility
  - Other risks relating to pregnancy e.g. cardiac toxicity
  - Uterine radiotherapy
- ✓ Time, expertise and funding availability

Validation of patients' selection in this field requires long-term studies following-up women to assess the number who achieved pregnancies with and without fertility preservation procedures against the criteria on which patients were selected. This aspect of the underpinning evidence base is very much in its infancy with reports of pregnancies following FP being generally case series with obvious difficulties regarding an appropriate control or comparison group. Data are however emerging comparing outcomes of oocyte vitrification and subsequent use in women who have stored oocytes for non-medical compared with oncological indications (Cobo *et al.*, 2018). This has also been attempted in relation to children offered ovarian tissue cryopreservation, but with POI as an outcome rather than infertility given the age of the girls included (Wallace *et al.*, 2014). Relevant data in adult women include identification of those who were able to achieve a pregnancy after a FP procedure without further medical intervention, i.e. without the use of their stored gametes or ovarian tissue and such data, albeit often incomplete, has been published by some centres (e.g. (Schmidt *et al.*, 2013)). Clearly, there is a need for accurate analyses of outcomes of women who have chosen to or not to proceed to FP to allow more informed patient decision-making. The analysis of the evidence available at the present time in subsequent sections will, we hope, stimulate high quality research in this aspect.

Specifically for FP, the following possible complications of FP procedures should be considered in patient assessment and appriate steps taken to prevent them:

- Anaesthetic complications (including cardiac issues)
- Thrombotic risk
- Infection risk, particularly in immunodeficient patients
- Complications from difficult access to ovaries (patient issues and/or disease-related)
- Complications of FP in patients with hormone-sensitive cancers

Recommendations

Patients require an individual assessment of the need and suitability of FP.	**GPP**

A multidisciplinary team is recommended to have an accurate assessment of risks	**GPP**

Justification

There will always be a balance between providing FP to patients at risk, and not providing when the risk is low. This is futher complicated by uncertainty over the risk when applied to an individual, and issues around the degree of invasiveness of the planned procedure, what risk it carries for the patient, and the likelihood of success-meaning a future successful pregnancy, in relation to that chance without the FP intervention.

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C2. Gonadotoxic treatments

Treatments for cancer and other medical conditions may cause gonadal damage by directly affecting the growing and non-growing ovarian follicle pool, the ovarian stroma or the blood supply to the ovary. Treatment-induced gonadotoxicity may lead to premature ovarian insufficiency (POI) defined as the absence of menstrual cycles for ≥4 months and elevated FSH levels in adult women of 40 years of age or younger (Webber *et al.*, 2016). Applying this definition to patients receiving gonadotoxic treatments may be problematic. On one hand, 4 months is a short timeframe for patients exposed to anticancer therapies; if menstrual function returns, it usually occurs within 1 year following treatment completion, but it can happen also more than 2 years following the end of therapy (Jacobson *et al.*, 2016). On the other hand, irrespectively of the development of POI, patients exposed to gonadotoxic therapies who resume menstrual function after treatment may experience other negative treatment-related consequences including infertility and early menopause (Letourneau *et al.*, 2012, Barton *et al.*, 2013).

For defining the risk of treatment-induced gonadotoxicity, it is important to highlight that the available studies on this regard have not used homogeneous definitions of POI so that comparisons between different treatments or even between studies focusing on the same therapy can be problematic. Treatment-induced gonadotoxicity has been assessed using amenorrhoea at different timepoints following completion of therapy in some studies, while others have applied composite endpoints for its definition (amenorrhoea and post-menopausal hormonal levels) (Lee *et al.*, 2006). Only limited evidence exists to estimate treatment-induced gonadotoxicity using other parameters (anti- Müllerian hormone [AMH] levels, antral follicle count [AFC] or, more importantly, post-treatment pregnancy and age at POI/menopause) which may reflect more properly the impact of the treatment on the ovarian reserve and fertility potential of the patients (Gracia *et al.*, 2012). Among ovarian reserve markers, AMH is considered more sensitive and relevant than FSH, LH, estradiol or inhibin B; therefore, most of the studies that assessed ovarian reserve markers to estimate treatment-induced gonadotoxicity have focused on AMH during and after treatment completion. This is reviewed in section C3. Ovarian reserve testing

PICO Question: Which factors should be taken into account when estimating the individual risk of gonadotoxicity for a certain patient?

Although different mechanisms of gonadotoxicity have been proposed for each class of chemotherapy agent (Morgan *et al.*, 2012, Bedoschi *et al.*, 2016, Codacci-Pisanelli *et al.*, 2017), it is the type and dose of chemotherapy that are the major factors determining risk of treatment-induced POI. Both oocyte and granulosa cells can be vulnerable to the toxic effect of chemotherapy; moreover, injury to blood vessels and focal ovarian cortical fibrosis are other potential consequences of cytotoxic therapy administration (Meirow *et al.*, 2007, Morgan *et al.*, 2012).

The gonadotoxicity of radiotherapy is dependent on the field of radiation, its dose and fractionation; radiotherapy can directly damage ovarian follicles and other ovarian tissues, but may also cause adverse effects on other reproductive organs, notably the uterus (Wallace *et al.*, 2003, Wallace *et al.*, 2005, Adriaens *et al.*, 2009, Wo and Viswanathan, 2009).

Apart from gonadotoxic treatments, the disease itself (e.g. lymphoma) or surgery (e.g. endometriosis) can be associated with gonadal damage and diminished ovarian reserve (Lawrenz *et al.*, 2012, Lekovich *et al.*, 2016, Horton *et al.*, 2019). The impact of surgery is reviewed in section C3. Ovarian reserve testing.

In terms of patient characteristics, age is the most important factor affecting the risk of gonadotoxicity (Letourneau *et al.*, 2012). Pre-treatment ovarian reserve, linked with age, is another

crucial factor, for which the evidence has been discussed in section C3. Ovarian reserve testing. Other patient-related factors that may potentially influence the risk of treatment-induced POI include hereditary factors, with most of the evidence on the impact of germline mutations in the *BRCA* genes (Lambertini *et al.*, 2017b, Peccatori *et al.*, 2018, Turan and Oktay, 2020).

Cancer

Breast cancer

Chemotherapy in premenopausal women with early breast cancer has a known gonadotoxic effect as shown in many studies reporting on rates of POI (mostly defined as treatment-induced amenorrhoea (Zhao *et al.*, 2014)) as well as impact on patients' ovarian reserve (measured by AMH levels (Anderson *et al.*, 2006)).

The highest risk of gonadotoxicity with the use of anticancer systemic therapies in early breast cancer patients is associated with the administration of the alkylating agent cyclophosphamide, commonly given as part of (neo)adjuvant chemotherapy regimens (see Table 7). Compared to chemotherapy not including this agent, cyclophosphamide-based regimens are associated with a significantly higher risk of POI, with more than double the chances of developing treatment-induced amenorrhoea (odds ratio [OR] 2.25; 95% CI 1.26–4.03) (Zhao *et al.*, 2014).

Anthracyclines and taxanes are two widely used classes of chemotherapy agents administered as part of (neo)adjuvant treatment in women with early breast cancer. The use of anthracycline-based or taxane-based regimens significantly increased the risk of treatment-induced amenorrhoea (OR 1.39; 95% CI 1.15-1.70 and OR 1.24; 95% CI 1.03-1.50, respectively) compared to regimens without anthracyclines or taxanes (Zhao *et al.*, 2014). Administering these agents with a dose-dense schedule[2] (i.e. every 2 weeks) versus a standard 3-weekly schedule was not associated with a higher risk of treatment-induced amenorrhoea (OR 1.00; 95% CI 0.80-1.25) (Lambertini *et al.*, 2017a).

With the administration of all these chemotherapy agents, AMH levels fall to undetectable levels in most women and generally persist at very low levels after treatment completion, with the extent of recovery determined by age and pre-treatment AMH levels (Anderson *et al.*, 2006, Su *et al.*, 2014, Freour *et al.*, 2017).

Currently, the two most common chemotherapy regimens used as (neo)adjuvant chemotherapy in early breast cancer are sequential treatment with an anthracycline plus cyclophosphamide followed by a taxane or the combination of cyclophosphamide plus a taxane (i.e. the TC regimen). Regarding the first combination, the addition of a taxane to anthracycline plus cyclophosphamide showed to adversely affect menses recovery (OR 2.04; 95% CI 1.25-3.33)[3] (Silva *et al.*, 2016). Consistent with this, a more recent retrospective analysis within a phase III trial reported an increased risk of treatment-induced amenorrhoea with the addition of a taxane to anthracycline-based chemotherapy (OR 1.92; 95% CI 1.44-2.56) (Lambertini *et al.*, 2019a). Sequential use of a taxane following anthracycline plus cyclophosphamide is also associated with reduced AMH levels 1 year following treatment completion (Lambertini *et al.*, 2019c). Another study reported similar rates of treatment-induced amenorrhoea with the TC regimen and a sequential regimen with anthracycline plus cyclophosphamide followed by a taxane (81% and 80% of patients reported cessation of menses after chemotherapy) (Ejlertsen *et al.*, 2017).

Targeted treatments

Limited evidence exists on the risk of treatment-induced gonadotoxicity associated with the use of targeted agents. The two studies reporting rates of treatment-induced amenorrhoea in patients

[2] i.e. using the same dose but given at a shorter interval between treatment cycles to increase the efficacy of chemotherapy

[3] The OR for menses recovery was calculated from the OR for treatment-induced amenorrhea presented in the paper by Silva and colleagues.

receiving chemotherapy with anthracycline- and/or taxane-based regimens plus the anti-HER2 agents trastuzumab and/or lapatinib have suggested likely gonadal safety of these agents (Ruddy *et al.*, 2015, Lambertini *et al.*, 2019b).

Endocrine treatments

The use of endocrine therapy is standard of care for patients with hormone receptor-positive breast cancer. There are three main approaches currently recommended, for a duration of 5 years (possibly prolonged to 10 years) with the choice based on patient individual risk of relapse: tamoxifen alone, GnRH analogue plus tamoxifen and GnRH analogue plus an aromatase inhibitor (Burstein *et al.*, 2016, Cardoso *et al.*, 2019).

Although tamoxifen following use of chemotherapy appears to increase the risk of amenorrhoea (OR 1.48; 95% CI 1.28-1.70) (Zhao *et al.*, 2014), there is no apparent negative effect of these agents on the ovarian reserve. Several studies have shown no difference in AMH levels between patients receiving tamoxifen following chemotherapy or not (Anderson *et al.*, 2017b, Dezellus *et al.*, 2017, Freour *et al.*, 2017, Lambertini *et al.*, 2019c). Nevertheless, GnRH analogue treatment can suppress AMH levels (Anderson *et al.*, 2006). Importantly, the ovarian function may recover during the use of an aromatase inhibitor alone in premenopausal women (even those beyond 45 years of age) that developed chemotherapy-induced amenorrhoea with potential negative consequences for treatment efficacy (van Hellemond *et al.*, 2017).

Patient-related factors

Among patient-related factors, age represents the most important factor influencing the risk of treatment-induced gonadotoxicity (Silva *et al.*, 2016). Depending on patients' age at the time of treatment, the same chemotherapy regimen can be associated with a high risk of gonadotoxicity (>80% chances of treatment-induced amenorrhoea) in patients older than 40 years and low risk (<20% chances of treatment-induced amenorrhoea) in patients younger than 30 years (Lee *et al.*, 2006, Lambertini *et al.*, 2016). Baseline ovarian reserve measured by AMH levels influences and predicts the risk of developing treatment-induced amenorrhoea (Anderson and Cameron, 2011, Silva *et al.*, 2016, Anderson *et al.*, 2017b, Dezellus *et al.*, 2017, Freour *et al.*, 2017). Hereditary conditions may also have a role; there is evidence suggesting a potential negative effect of carrying germline *BRCA* mutations on baseline ovarian reserve and performance of fertility preservation strategies in young breast cancer patients (Titus *et al.*, 2013, Lambertini *et al.*, 2018, Turan *et al.*, 2018). However, the limited data reporting on chances of treatment-induced POI (defined based on amenorrhoea rates (Valentini *et al.*, 2013) or AMH levels (Lambertini *et al.*, 2019c) following therapy completion) have not shown any apparent increased risk for *BRCA*-mutated breast cancer patients as compared to those without mutations. The impact of other anthropometric and lifestyle factors (including body mass index and smoking history) and a potential role of genetic variants (single nucleotide polymorphisms) on the risk of treatment-induced gonadotoxicity remains to be clarified (Abusief *et al.*, 2012, Ruddy *et al.*, 2019).

Haematological cancers

The use of chemotherapy in premenopausal women with haematological cancers has a known gonadotoxic effect as reported in several studies assessing POI rates (mostly defined as treatment-induced amenorrhoea (Overbeek *et al.*, 2017)) and impact on patients' ovarian reserve (measured by AMH levels (Peigne and Decanter, 2014)). The largest amount of data is available for patients with lymphoma (Overbeek *et al.*, 2017).

In Hodgkin lymphoma, chemotherapy regimens can include alkylating agents [MOPP, MOPP/ABV hybrid, RSQB, BEACOPP] or not [ABVD, EBVP], and this is considered the main determinant of gonadotoxic risk (see Table 7). The cumulative POI risk with the use of alkylating-based chemotherapy was 60% while it was only 3% for women exposed to non-alkylating regimens (age-adjusted hazard ratio [HR] 12.31; 95% CI 5.90-25.68) (van der Kaaij *et al.*, 2012). A linear dose-response relationship between alkylating chemotherapy and occurrence of POI was observed (HR per cycle

of alkylating chemotherapy 1.50; 95% CI 1.37-1.64). The risk of POI increased by 23% per year of age at the time of treatment; the effect of age was smaller in patients exposed to alkylating chemotherapy than in those treated with non-alkylating regimens (van der Kaaij *et al.*, 2012). Another study assessing ovarian function after early-Hodgkin lymphoma treatment showed recovery of regular menstrual cycles (mostly within 12 months) in more than 90% of women (Behringer *et al.*, 2013). However, in women receiving the BEACOPP regimen aged ≥ 30 years, the risk of POI increased significantly with 45% reporting amenorrhoea. In terms of impact on patients' ovarian reserve, a decrease in AMH levels is observed during both ABVD and BEACOPP regimens (Anderson *et al.*, 2018b). At one year after ABVD completion, AMH levels had returned to pre-treatment concentrations with no changes at longer follow-up. However, age strongly affected the extent of AMH recovery after ABVD: full recovery was observed in women younger than 35 years, with only partial recovery in patients ≥35 years. In patients treated with BEACOPP, there was very little recovery in AMH levels overall, with further increased risk of POI in patients older than 35 years (Anderson *et al.*, 2018b).

More limited evidence exists for patients with non-Hodgkin lymphoma. A retrospective analysis conducted within two trials assessed ovarian function and ovarian reserve of patients treated with CHOP or CHOEP chemotherapy regimens (see Table 7) (Meissner *et al.*, 2015). As compared to the general population, last menstrual bleeding occurred earlier in patients exposed to CHOP-like chemotherapy (47 years vs. 51 years). In patients without menstrual function and those older than 42 years, AMH was undetectable. In women younger than 42 years and with active menstrual function, AMH levels were decreased when compared with those expected in the general population of similar age (Meissner *et al.*, 2015).

Patients with haematological cancers treated with stem cell transplantation are likely to receive conditioning regimens with high-dose chemotherapy including alkylating agents with or without radiation therapy. Permanent POI and infertility are highly prevalent, even in the absence of total body irradiation (Tauchmanova *et al.*, 2003, Hammond *et al.*, 2007, Akhtar *et al.*, 2015). Women undergoing allogeneic or autologous stem cell transplantation have a high (>80%) risk of POI, with age of the patient at the time of transplantation and number of chemotherapy cycles being important predictors of ovarian function recovery (Tauchmanova *et al.*, 2003, Akhtar *et al.*, 2015). As compared to patients who receive autologous stem cell transplantation, gonadal toxicity may be worsened by an altered immunomodulation in the allogeneic setting (Akhtar *et al.*, 2015). Higher rates of menstrual function recovery (63%) have been recently reported in patients who underwent high-dose chemotherapy and autologous stem transplantation for non-Hodgkin and Hodgkin lymphoma with a median age of 25 years at the time of treatment (Akhtar *et al.*, 2015). The high gonadotoxicity of these regimens is also confirmed by the significant drop in AMH levels after treatment exposure (Di Paola *et al.*, 2013, Peigne and Decanter, 2014).

Very limited evidence exists on the gonadotoxicity of targeted therapies hence no conclusions can be drawn on their gonadotoxic impact (Gharwan *et al.*, 2016).

It should be noted that, in addition to type of chemotherapy regimen and age at the time of treatment, evidence exists on a potential negative effect of the disease itself on baseline ovarian reserve and performance of FP strategies in women with lymphoma (Lawrenz *et al.*, 2012, Lekovich *et al.*, 2016). However, it is unknown whether and to what extent the disease itself may contribute to increasing the risk of treatment-induced gonadotoxicity.

Table 7 Risk of treatment-induced gonadotoxicity in cancer patients associated with the main systemic anticancer therapies

	RISK CATEGORY	TYPE OF ANTICANCER TREATMENT
	High risk **(> 80% risk of treatment-induced amenorrhoea)**	• Cyclophosphamide-based regimens (with anthracyclines and/or taxanes: (F)EC/(F)AC alone or followed by T or P, TC) in breast cancer patients aged ≥ 40 years • Conditioning regimens for HSC transplantation with cyclophosphamide and/or TBI in patients with haematological cancers • Abdominal and pelvic radiotherapy to a field that includes the ovaries
	Intermediate risk **(40%-60% risk of treatment- induced amenorrhoea)**	• Cyclophosphamide-based regimens (with anthracyclines and/or taxanes: (F)EC/(F)AC alone or followed by T or P, TC) in breast cancer patients aged 30-39 years • Alkylating agent-based regimens (e.g. MOPP, RSQB, BEACOPP, CHOP, CHOPE) in lymphoma patients
	Low risk **(< 20% risk of treatment-induced amenorrhoea)**	• Cyclophosphamide-based regimens (with anthracyclines and/or taxanes: (F)EC/(F)AC alone or followed by T or P, TC) in breast cancer patients aged ≤ 30 years • Non-alkylating agent-based regimens (e.g. ABVD or EBVP) in lymphoma patients aged ≥ 32 years • BEP / EP in patients with non-epithelial ovarian cancers • FOLFOX, XELOX or capecitabine in patients with colorectal cancers • Multi-agent chemotherapy (EMA-CO and platinum-based combinations) for gestational trophoblastic tumours • Radioactive iodine (I-131) in patients with thyroid cancer
	Very low or no risk	• Targeted agents (trastuzumab, lapatinib and rituximab) ? • Tamoxifen and GnRH analogue • Non-alkylating agent-based regimens (e.g. ABVD or EBVP) in lymphoma patients aged < 32 years • Single-agent methotrexate
	Unknown risk	• Platinum- and taxane-based chemotherapy in patients with gynaecological and lung cancers • Majority of targeted therapies (monoclonal antibodies and small molecules like tyrosine kinase inhibitors) and immunotherapeutic agents

Abbreviations: (F)EC/(F)AC = 5-fluoruracil, epirubicin, doxorubicin, cyclophosphamide; T = docetaxel; P = paclitaxel; GnRH analogue = gonadotropin releasing hormone analogue; HSC = hematopoietic stem cell; TBI = total body irradiation; MOPP = mechlorethamine, vincristine, procarbazine, prednisone; RSQB or MOPP/ABV hybrid, = MOPP/doxorubicin, bleomycin, vinblastine; BEACOPP = cyclophosphamide, doxorubicin, vincristine, bleomycin, etoposide, procarbazine, prednisone; ABVD = doxorubicin, bleomycin, vinblastine, dacarbazine; EBVP = epirubicin, bleomycin, vinblastine, prednisone; CHOP = cyclophosphamide, doxorubicin, vincristine, prednisone; CHOPE = CHOP plus etoposide; BEP = etoposide, cisplatin, bleomycine; EP = etoposide, cisplatin; FOLFOX = 5-fluoruracil, oxaliplatin; XELOX = capecitabine, oxaliplatin; EMA-CO = etoposide, actinomycin D, methotrexate followed by cyclophosphamide and vincristine;

Gynaecological cancers

For gynaecological cancers, surgical procedures (including hysterectomy and bilateral salpingo-oophorectomy) have a direct effect on female reproductive potential (as discussed in section C3. Ovarian reserve testing). In addition to surgery, these patients are treated with pelvic radiotherapy and/or chemotherapy, which can further increase the risk of gonadotoxicity.

As oocytes are highly sensitive to ionizing radiation, abdominal and pelvic radiotherapy are associated with a significant risk of gonadotoxicity (Chan and Wang, 2017). Age of the patient at the time of treatment and cumulative dose of radiotherapy are the crucial factors, with a smaller sterilizing dose needed for POI development with increasing age at the time of treatment. Other important factors are the number and magnitude of fractions and the size of the radiation field. Due to scatter radiation, it may not be always easy to determine the exact dose reaching the ovaries (Chan and Wang, 2017). According to the dose of radiation, age of the patient and specific site of treatment, pelvic radiotherapy can cause injury to the uterus with subsequent potential risk of pregnancy-related complications (see E2. Obstetric outcomes).

The most common chemotherapy regimen used for the treatment of gynaecological cancers (epithelial ovarian cancer and cervical cancer) includes the combination of a platinum agent (e.g. carboplatin) and a taxane (e.g. paclitaxel). There is currently a lack of robust data to counsel patients on the risk of gonadotoxicity associated with this combination. A recent study assessed ovarian function and reproductive outcomes in patients with ovarian cancer undergoing fertility-sparing treatment and chemotherapy (Ceppi *et al.*, 2019). Among the 73 patients with epithelial ovarian cancer exposed to adjuvant chemotherapy, the majority received single-agent cisplatin or carboplatin with only 4 patients exposed to carboplatin plus paclitaxel. No apparent negative effect of chemotherapy exposure was observed (Ceppi *et al.*, 2019). However, no strong conclusions can be derived specifically on the potential gonadotoxicity of the combination treatment with a platinum agent and a taxane.

BEP- or EP-chemotherapy regimens are often used for the treatment of non-epithelial ovarian cancers. A case-control study reported a high likelihood of retaining ovarian function and fertility after treatment in a young population of patients exposed to fertility-sparing surgery and (in most cases) BEP chemotherapy (Gershenson *et al.*, 2007). However, a more recent study has shown a potential increased risk of treatment-induced amenorrhoea and earlier spontaneous menopausal age after this same treatment; nevertheless, high conception rates were reported (Ceppi *et al.*, 2019).

Overall, chemotherapy regimens used for treating young women with gynaecological cancers can be considered associated with a low risk of gonadotoxicity. This risk varies significantly according to the type of chemotherapy agent, to the dose and length of exposure, and to the patient's age at the time of treatment (Chan and Wang, 2017). More data are needed to properly define the risk of gonadotoxicity associated with the combination treatment of a platinum agent and a taxane.

Other cancers

Data on the risk of gonadotoxicity with the use of anticancer systemic therapies in patients with malignancies other than breast, haematological and gynaecological cancers are more limited (Overbeek *et al.*, 2017). For all cancer types, the two most important risk factors influencing the risk of treatment-induced gonadotoxicity are use of alkylating agents and older age at the time of treatment (Overbeek *et al.*, 2017).

Osteosarcoma and Ewing sarcoma are rare cancers in adults but more common in paediatric and adolescent patients. The rates of treatment-induced amenorrhoea in survivors of osteosarcoma and Ewing sarcoma treated with anthracycline- and cyclophosphamide-based chemotherapy regimens with or without radiotherapy range between 3% and 25% (Longhi *et al.*, 2012, Overbeek *et al.*, 2017). Predisposing factors for higher risk of permanent amenorrhoea were older age, use of high-dose chemotherapy and radiotherapy (Longhi *et al.*, 2012) .

Surgery, radiotherapy and chemotherapy are important components in the management of patients diagnosed with colorectal cancer, which is increasingly common in young women. Overall,

this diagnosis results in reduced chance of subsequent pregnancy (standardized incidence ratio [SIR] 0.53; 95% CI 0.43-0.64) (Anderson *et al.*, 2018a). While no apparent negative effect on female reproductive function and fertility is expected with surgical resection for colon cancer, potential negative consequences cannot be excluded with resections below the peritoneal reflection (Spanos *et al.*, 2008). Neoadjuvant (or adjuvant) chemoradiation is an important part of the treatment in patients with rectal cancer. Although proper evidence is lacking to counsel young women on the gonadotoxicity of this approach, pelvic radiotherapy is known to potentially lead to POI and infertility, with its gonadotoxicity risk being strongly influenced by the dose and field of radiation as well as the age of the patients at the time of treatment (Spanos *et al.*, 2008). Fluoropyrimidines (5-fluoruracil and capecitabine) are the backbone chemotherapy agents for patients with colorectal cancer. While these agents are associated with a low risk of gonadotoxicity, their combination with oxaliplatin may be more harmful (Spanos *et al.*, 2008). Two retrospective studies have assessed the gonadotoxicity of the most commonly used regimens in this setting: FOLFOX, XELOX or capecitabine alone (Cercek *et al.*, 2013, Wan *et al.*, 2015). The rate of amenorrhoea ≥ 1 year following chemotherapy completion was low (4%-16%) (Cercek *et al.*, 2013, Wan *et al.*, 2015). A trend for higher risk of amenorrhoea was observed in patients older than 40 years (Cercek *et al.*, 2013). In women with rectal cancer exposed to chemoradiotherapy, the rate of amenorrhoea was 94.1% (Wallace *et al.*, 2003).

Gestational trophoblastic tumours are a spectrum of rare pregnancy-related disorders that include the malignant disorders choriocarcinoma and placental-site trophoblastic tumour. Low-risk patients receive single-agent chemotherapy, either with methotrexate or actinomycin D; high-risk patients receive multi-agent chemotherapy consisting of etoposide, actinomycin D and methotrexate followed by cyclophosphamide and vincristine (EMA-CO regimen), or other platinum-etoposide combinations (EMA-EP, BEP or VIP and ICE including ifosfamide) in resistant patients. In the two studies reporting risk of gonadotoxicity with these regimens, rates of early menopause varied considerably based on chemotherapy regimen and age at the time of treatment (Savage *et al.*, 2015, Cioffi *et al.*, 2018). Single-agent methotrexate had no detectable effect on early menopause (Savage *et al.*, 2015), although 33% of women reported temporary amenorrhoea during treatment (Cioffi *et al.*, 2018). Among women who received multi-agent chemotherapy, rates of early menopause were 13% and 36% by age 40 and 45 years (Savage *et al.*, 2015). A total of 57.1% and 36.4% of patients treated with single-agent or multi-agent chemotherapy had a pregnancy following treatment completion, respectively (Cioffi *et al.*, 2018).

The treatment of differentiated thyroid carcinoma consists of surgery (total or near-total thyroidectomy) followed by treatment with radioactive iodine (I-131) in high-risk patients and in selected low-risk patients. In a systematic review, all women resumed regular menstrual cycles within 1 year following treatment completion with normalization of FSH levels (Clement *et al.*, 2015). Nevertheless, two small studies reported a potential negative effect of I-131 therapy on patients' ovarian reserve, with a significant decrease in AMH levels after treatment and only partial subsequent recovery (Evranos *et al.*, 2018, Yaish *et al.*, 2018). A trend for reduced AMH levels after I-131 therapy was also shown in another study (Giusti *et al.*, 2018). Younger age at menopause was described for patients with DTC who received I-131 therapy compared to those not exposed to this treatment (49.5 years vs. 51.0 years) (Clement *et al.*, 2015). The pregnancy rate appears not to be affected by I-131 therapy administration (Clement *et al.*, 2015, Giusti *et al.*, 2018) although overall, women treated for thyroid cancer have a reduced chance of post-treatment pregnancy (Anderson *et al.*, 2017a).

A recent small study has investigated the risk of amenorrhoea in patients with lung cancer (Cathcart-Rake *et al.*, 2019). Among the 182 patients included (with a median age of 43 years), 85 received chemotherapy consisting of platinum salts in all cases, with a taxane in most of them. The majority of patients (64%) developed chemotherapy-induced amenorrhoea; out of the 3 patients exposed to targeted therapy alone, 2 remained premenopausal (Cathcart-Rake *et al.*, 2019). More data are needed to properly define the risk of gonadotoxicity with the therapies currently available for the management of lung cancer.

The risk of gonadotoxicity associated with the use of targeted agents and immunotherapy is largely unknown. These treatments are already standard of care (BRAF and MEK inhibitors and immune checkpoint inhibitors in melanoma) or they are currently under investigation in the curative setting for a range of malignancies. Therefore, there is an urgent need to investigate their impact on ovarian function, ovarian reserve and fertility potential of cancer patients to allow accurate counselling on their potential gonadotoxicity risk.

Benign diseases

The risk of gonadotoxicity in patients with benign diseases is mainly due to treatments with high cumulative doses of alkylating agents given as immunosuppressive therapy. Fertility preservation may be challenging in these patients due to severe health conditions, long-term therapy (i.e. hydroxyurea), high risk of thrombosis and/or the genetic context (Condorelli and Demeestere, 2019)

Autoimmune diseases

Severe manifestations of autoimmune diseases such as systemic sclerosis, Wegener granulomatosis, systemic lupus erythematosus (SLE) and antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis may require immunosuppressive therapy with daily oral doses (0.5-2mg/kg/day) or intravenous pulse (0.5-1 g/m2/pulse) of cyclophosphamide. Although the daily dose is low compared to the intravenous dose, oral treatment can be administered for several months, leading to a high cumulative dose. In a study including 67 pre-menopausal patients treated with daily cyclophosphamide for vasculitis, almost 50% of the patients developed treatment-induced POI (Tuin *et al.*, 2016). The risk of POI was two times higher when patients received a total dose above 16.6 g compared to those who received a lower dose (OR 2.60; 95% CI 1.38-4.90) (Tuin *et al.*, 2016). In another study of 42 patients diagnosed with granulomatosis before the age of 50 years, the decline in the ovarian reserve was inversely correlated with the cumulative dose of cyclophosphamide, with a decrease of 0.74 ng/ml in AMH level for every 10 g of cyclophosphamide (Clowse *et al.*, 2011). Modest restoration of AMH levels could be observed after treatment. No difference was reported between intravenous and oral cyclophosphamide therapy in premenopausal patients with SLE; treatment-induced POI was observed in 39% of the patients below the age of 30 years and 59% in those between 30 and 40 years (Manger *et al.*, 2006).

Other immunosuppressive treatments such as mitoxantrone have also been associated with gonadotoxicity. In a study including 189 patients treated with mitoxantrone before the age of 45 years for multiple sclerosis, the authors reported 26% incidence of post-treatment amenorrhoea, with an increased risk of 2% per mg/kg of cumulative dose (Cocco *et al.*, 2008). In a large cohort of 371 women treated with mitoxantrone, the rate of treatment-induced permanent amenorrhoea was 17.3%; no cases were reported among patients treated before the age of 25 years (Le Page *et al.*, 2011).

In addition to the effects of treatment, the disease itself may also impact the ovarian reserve. Lower AMH levels have been reported in patients with autoimmune diseases such as vasculitis, rheumatoid arthritis (RA) or SLE without chemotherapy exposure (Morel *et al.*, 2013, Bermas and Sammaritano, 2015, Brouwer *et al.*, 2015). (see also section C3. Ovarian reserve testing)

Benign haematological diseases

Haematopoietic stem cell transplantation (HSCT) remains the only curative option for several benign haematological diseases such as thalassemia, sickle cell disease, aplastic anaemia, Fanconi anaemia or myeloproliferative syndromes. Although it is usually proposed during childhood, adults may also benefit from this treatment. A conditioning regimen for HSCT includes high dose alkylating agents and is associated with high risk of permanent amenorrhoea (see section

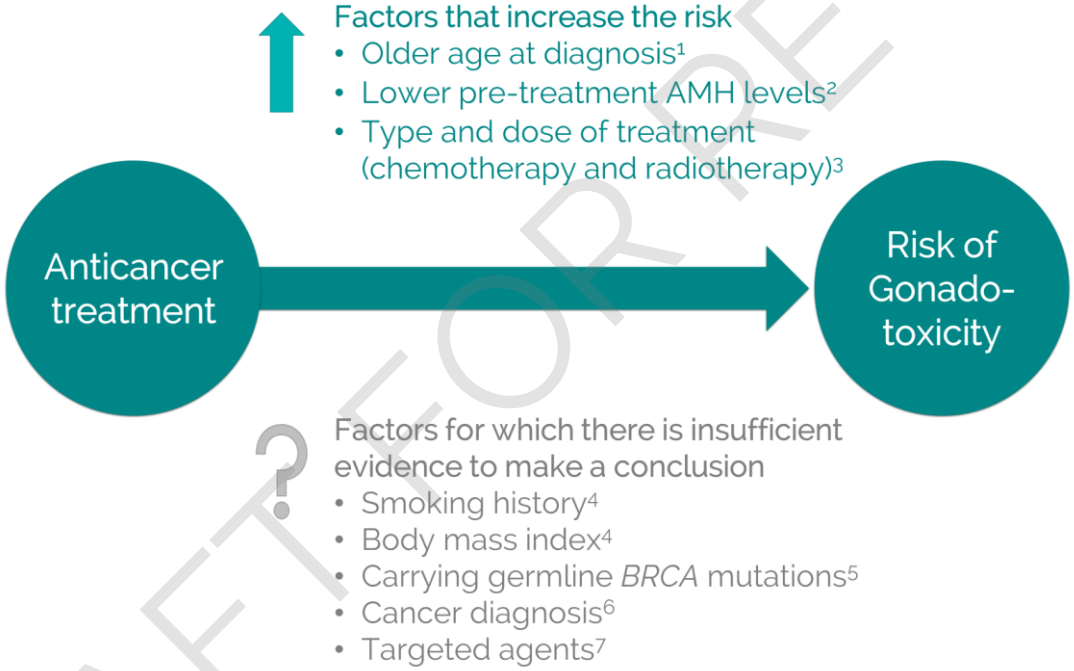
Haematological cancers). Recent reduced-intensity chemotherapy, based on fludarabine and melphalan or treosulfan, has been proposed to reduce the toxicity of standard conditioning regimen but the gonadotoxicity remains to be investigated (Condorelli and Demeestere, 2019).

Long-term treatment with hydroxyurea as well as iron overload secondary to repeated transfusions may also negatively impact on the ovarian reserve and fertility potential of patients with haematological benign diseases (Elchuri *et al.*, 2015, Condorelli and Demeestere, 2019).

Benign gynaecological diseases

All benign conditions that involve the ovaries such as endometriosis, ovarian cysts or borderline tumours may be at risk of infertility due to the disease itself or surgical-related depletion of the ovarian reserve (Condorelli and Demeestere, 2019) (see also section C3. Ovarian reserve testing).

Figure 3 Summary of factors to be considered when estimating the risk of gonadotoxicity.



Supporting studies/reviews

[1]*Breast cancer,(Silva et al., 2016); Hodgkin Lymphoma, (van der Kaaij et al., 2012)*

[2]*Breast cancer, (Anderson and Cameron, 2011, Silva et al., 2016, Anderson et al., 2017b, Dezellus et al., 2017, Freour et al., 2017) (AMH) (evidence in BC)*

[3]*Breast cancer, (Lambertini et al., 2017a); Hodgkin Lymphoma, (van der Kaaij et al., 2012); Haematological cancers, (Tauchmanova et al., 2003, Akhtar et al., 2015)*

[4]*Breast cancer, (Abusief et al., 2012)*

[5] *(Valentini et al., 2013, Lambertini et al., 2019c)*

[6]*Lymphoma, (Lawrenz et al., 2012, Lekovich et al., 2016)*

[7]*Breast cancer (endocrine therapies), (Bernhard et al., 2007, Anderson et al., 2017b, Dezellus et al., 2017, Freour et al., 2017, Lambertini et al., 2019c); Breast cancer (anti-Her2), (Ruddy et al., 2015, Lambertini et al., 2019a)*

Recommendations

The risk of gonadotoxicity should be assessed in all patients undergoing anticancer treatments.	**GPP**

To estimate the individual risk of gonadotoxicity, the characteristics of the proposed treatment, the patient and the disease should be considered.	**STRONG** ⊕⊕○○

Justification

The evidence on the risk of treatment-induced gonadotoxicity relies mostly on retrospective and prospective cohort studies or secondary/exploratory analyses of randomized trials. Some of these studies have been summarized in systematic reviews and meta-analyses. Notably, treatment-induced gonadotoxicity has not been defined homogeneously in the available studies so that comparisons and strong conclusions are often difficult. While most of the studies have assessed treatment-related gonadotoxicity by using amenorrhoea rates following completion of therapy, there is limited evidence with the use of other markers (like AMH, AFC, age at menopause, pregnancy rates) that reflect more properly the treatment effect on the ovarian reserve and fertility potential of the patients. Nevertheless, consistent results from these studies have shown that age (strongly linked to pre-treatment ovarian reserve) and type/dose of treatment are the crucial factors impacting the risk of treatment-induced gonadotoxicity. Irrespective of the risk, all patients should be counselled about the gonadotoxicity of the proposed therapy to make fully informed decisions on the treatment and the possibility to access the available strategies for ovarian function and/or fertility preservation before its initiation.

Research recommendation

To investigate the impact of newer anti-cancer treatments (including targeted agents and immunotherapy) on ovarian function, ovarian reserve and fertility potential of cancer patients should be considered a research priority.

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C3. Ovarian reserve testing

Ovarian reserve reflects the quantity and quality of follicles in the ovaries and therefore depicts ovarian functionality at a given point in time (Iwase *et al.*, 2014). Ovarian reserve status is related to response to ovarian stimulation and reduced fertility and can be a useful surrogate marker for fertility potential.

Ovarian reserve tests include radiological (ultrasound of antral follicle count (AFC) and mean ovarian volume) and biochemical assessments (anti-Müllerian hormone (AMH), estradiol (E2) and follicle-stimulating hormone (FSH)). Although many ovarian reserve tests are currently being used in the clinic, AFC and serum levels of AMH appear to be the most promising markers mainly due to their low intercycle variation and ease of measure (Dewailly *et al.*, 2014). According to the Bologna criteria for the definition of "poor ovarian response" (POR), cut-off levels for AFC are less than 5 to 7 follicles, and AMH levels below 0.5-1.1 ng/ml (Ferraretti *et al.*, 2011).

Many diseases (such as cancer and autoimmune diseases), treatments (such as chemotherapy) or interventions (such as gender reassignment surgery) have been shown to affect the fertility potential in premenopausal women (Table 8). As these populations are at higher risk of low ovarian reserve at time of diagnosis or after treatment/intervention, ovarian reserve testing could be used to guide decisions for fertility preservation either at the time of diagnosis or before treatment or intervention.

Therefore, the aim of this PICO question is to provide evidence-based recommendations on the relevance of ovarian reserve testing at diagnosis or before treatment for each patient group.

PICO Question: Is it relevant to do ovarian reserve testing, and for whom?

Cancer

Early breast cancer and haematological malignancies

The most prevalent malignancies affecting post pubertal female patients are breast cancer and haematological malignancies (Hancke *et al.*, 2011, Fidler *et al.*, 2017). Whether these malignancies affect ovarian function per se, is still a matter of debate.

Some studies have shown that patients with haematological malignancies have reduced AMH levels in comparison to healthy controls (Lawrenz *et al.*, 2012, Dunlop and Anderson, 2015, Lekovich *et al.*, 2016). Other studies have shown that AMH levels may be lower in breast cancer patients older than 37 years of age, compared to healthy controls (Su *et al.*, 2013). The potential role of mutations in the BRCA1/2 genes with regards to the serum levels of AMH is still unclear. Whereas some studies have shown that carriers of BRCA1 and BRCA2 mutations have lower AMH levels than non-carrier controls (Titus *et al.*, 2013), others have not been able to confirm this observation (Van Tilborg *et al.*, 2016). Along these lines, a recent study has found that young breast cancer patients with BRCA1/2 mutations present lower pre-treatment AMH levels than patients with no mutations (Son *et al.*, 2019).

Similarly, ovarian stimulation outcomes for oocyte cryopreservation in cancer patients might be weaker than in infertile patients (Domingo *et al.*, 2012), although contrasting studies indicate no differences in number of oocytes retrieved in untreated cancer patients in comparison to healthy controls (Quintero *et al.*, 2010, Moraes *et al.*, 2019). A more recent study has found that lower AFC and AMH levels can be associated to lower primordial follicle density and number of *in vitro* matured oocytes in breast cancer patients (Grynberg *et al.*, 2019). Collectively, these data show that ovarian function might already be impaired in these patients already before any type of cancer treatment and that pre-treatment AMH levels might be correlated to ovarian stimulation response.

In addition, these patients undergo gonadotoxic cancer treatments which can result in premature ovarian insufficiency, amenorrhoea and infertility (Blumenfeld *et al.*, 2002, Lutchman Singh *et al.*, 2005, Ceppi *et al.*, 2019)

Several studies have investigated factors that affect ovarian recovery after chemotherapy and concluded that serum AMH levels taken before the start of chemotherapy treatment predict post-treatment recovery of ovarian function (Peigne and Decanter, 2014, Silva *et al.*, 2016, Dezellus *et al.*, 2017). These results provide a basis to support preservation of ovarian function or fertility prior to gonadotoxic treatment. Two studies have shown that pre-treatment AMH levels lower than 1.9ng/ml lead to long-term (longer than 5 years) loss of ovarian function (Anderson *et al.*, 2013, Dillon *et al.*, 2013), whereas another study found that patients with pre-treatment AMH levels lower than 0.7ng/mL experienced a significantly longer time to return of ovarian function (as measured by recovery of menses) (Su *et al.*, 2014).

A narrative review concluded that taking into consideration age and body mass index (BMI) together with AMH levels can increase the accuracy of the prediction of cancer-related ovarian failure (Dunlop and Anderson, 2015), based on a study reporting that women with high pre-treatment AMH and low FSH levels, younger age (<40 years old), or high BMI (>25kg/m^2) were more likely to regain ovarian function (Su *et al.*, 2014). Similar studies have collectively led to the development of several scoring systems for breast cancer patients that evaluate the risk of ovarian insufficiency (Anderson *et al.*, 2013, Su *et al.*, 2014, Barnabei *et al.*, 2015). Further studies are necessary to design similar prognostic tools for the other malignancies.

The interpretation of these studies in breast cancer and haematological malignancies should, however, be made with caution, as the primary outcome of those studies has solely been amenorrhoea and therefore there is little evidence that pre-treatment AMH levels can be used to predict post-treatment fertility in breast cancer patients. Only one retrospective study with breast cancer patients has shown that pre-treatment AMH levels are not associated to the occurrence of pregnancy (Hamy *et al.*, 2016).

Recommendations

Pre-treatment ovarian function, in particular through AMH levels, in premenopausal women with a diagnosis of breast cancer or haematological malignancy is a relevant predictor of post-treatment recovery of ovarian function (evaluated as recovery of menses).	**STRONG** ⊕⊕○○

For patients in whom you want to know fertility status, the value of pre-treatment AMH levels for predicting post-treatment fertility is unclear.	**WEAK** ⊕○○○

Age, pre-treatment AMH levels, as well as proposed gonadotoxic treatment type and dose, should be taken into consideration when estimating the risk of post-treatment POI.	**STRONG** ⊕○○○

Justification

There is evidence showing that pre-treatment ovarian reserve (measured by AMH levels) is correlated with recovery of ovarian function after gonadotoxic treatment. For prediction of fertility or chance of pregnancy, pre-treatment AMH levels seem to be less relevant, although evidence for this is very limited. Additional studies assessing ovarian reserve and reproductive outcomes after cancer treatment are highly warranted.

Studies have shown that apart from AMH levels, other factors can affect post-treatment ovarian function. This recommendation stresses the importance of considering multiple factors when estimating risk of post-treatment POI and/or infertility, rather than making an estimation solely on pre-

treatment AMH levels. This recommendation is based on indirect evidence of other factors affecting post-treatment ovarian function and the general limitations of AMH assessment.

Other malignancies

There is no evidence supporting the role of ovarian testing to guide decisions on fertility preservation in patients with other types of malignancies. Studies have provided evidence that levels of serum AMH are affected after chemotherapeutic treatment in several malignancies, including Wilms tumours, Ewing sarcomas, gliomas, osteosarcomas (Iwase *et al.*, 2015). One prospective cohort study included in the review, investigated 46 women with varying types of neoplasias (but including 19 breast cancer patients) and reported that those with lower pre-treatment AMH levels (<2ng/ml) showed a slower rate of recovery of ovarian function (as measured by post-treatment AMH levels) (Dillon *et al.*, 2013). Whether these post-treatment AMH levels correlate to ovarian insufficiency or infertility is not known, and therefore the relevance of ovarian reserve testing for malignancies, other than breast cancer or haematological malignancies is still uncertain.

Recommendation

Pre-treatment ovarian reserve testing in women with malignancies (other than breast or haematological cancer) is likely to be of high relevance, based on the indirect evidence from breast and haematological cancers.	**WEAK** ⊕◯◯◯

Justification

This recommendation is based on the same evidence and considerations as for breast cancer and haematological cancers, although supported by the limited data available specifically for these other cancers (Dillon et al., 2013).

Benign diseases

Although two completely different entities, systemic lupus erythematosus (SLE) and endometriosis share an important feature: both the disease per se as well as its treatment have a negative impact of the fertility of the patients.

Systemic lupus erythematosus

Many studies have reported adverse reproductive outcomes in women with SLE (Oktem *et al.*, 2016) . Pre-treatment AMH levels, AFC and ovarian volume are decreased, whereas FSH and LH are increased in comparison to healthy controls (Lawrenz *et al.*, 2011). Although menstrual irregularities are associated with the disease activity (Shabanova *et al.*, 2008), no correlation was found between AMH levels and disease activity (Lawrenz *et al.*, 2011). This suggests that patients with SLE already present poor ovarian reserve and function regardless of the activity of the disease or exposure to SLE therapy.

Cytotoxic immunosuppressive agents such as mycophenolate, azathioprine, methotrexate (MTX), or cyclophosphamide (CP) are indicated in the treatment of serious complications of SLE. Several meta-analyses (based on similar studies) have concluded that exposure to CP exerts an important negative impact on ovarian function, as measured by AMH levels (Mak *et al.*, 2009, Liu *et al.*, 2012, Henderson *et al.*, 2013). In fact, several studies summarized in a narrative review have shown that exposure to CP is the most significant risk factor for the development of ovarian insufficiency in SLE patients, with duration of treatment and cumulative dose as most important parameters (Oktem *et al.*, 2016). Although MTX has been historically considered a safer treatment with regards to ovarian function, a study from 2014 has shown an inverse correlation between cumulative MTX dose and AMH levels (de Araujo *et al.*, 2014). High doses of MTX were shown to lead to decreased AMH levels, although the number of patients in this study was limited and therefore, further large-scale studies need to be performed to validate these results.

Surprisingly, the role of ovarian testing (in particular AMH levels) in predicting the probability of subsequent pregnancy in SLE patients is still questionable. A cohort study found that the risk of failure to conceive (natural conception) in SLE patients was not associated with AMH levels but rather to cumulative CP dose and older age (Morel *et al.*, 2013). In this study, a cumulative dose of 17 grams and age over 38 years was associated with failure to conceive.

Recommendation

The relevance of ovarian reserve testing to help guide fertility preservation options or treatment decisions in SLE patients is low.	**WEAK** ⊕○○○

Justification

SLE and SLE treatment, in particular cyclophosphamide, results in a decrease of AMH levels and a reduced response to ovarian stimulation. Although indicative of ovarian function, ovarian reserve testing in SLE patients does not seem to be associated to fertility outcomes (natural conception), and women with SLE and low AMH levels might still become pregnant.

The impact of these cytotoxic immunosuppressive agents on ovarian reserve is dependent on duration of treatment and cumulative dose (see also Error! Reference source not found.). For women undergoing t reatment with high doses of CP, fertility preservation could be an option (irrespective of AMH levels). However, patients need to be informed of the limitations of FP and possible contraindications for a future pregnancy.

Endometriosis

Ovarian endometriomas are cysts that release potentially toxic compounds which diffuse through the cyst wall and damage the ovarian reserve (Muzii *et al.*, 2018). Several studies (Kasapoglu *et al.*, 2018, Ashrafi *et al.*, 2019) and a recent meta-analysis of 17 studies with 968 patients with endometrioma (Muzii *et al.*, 2018), have found that AMH levels are decreased in unoperated patients with endometriomas in comparison to healthy controls. Involvement of the ovaries seems to have a role in this, since AMH levels in patients with bilateral endometriomas in comparison to patients with unilateral endometriomas are lower (Karadag *et al.*, 2019, Younis *et al.*, 2019).

Similarly, the number of oocytes retrieved during *in vitro* fertilization procedures are also affected in unoperated endometriomas patients (Inal *et al.*, 2019), an effect that is more relevant in patients with bilateral endometriomas (Reinblatt *et al.*, 2011, Benaglia *et al.*, 2013). Interestingly, AFC seems to be a better marker of ovarian reserve than AMH levels in serum in women undergoing IVF (Inal *et al.*, 2019), as AFC and not AMH levels was correlated to a reduction in the number of oocytes retrieved.

Conflicting reports exist regarding the relation between AMH levels and endometrioma size and, thus, doubts of the relevance of endometrioma size on ovarian reserve still remain (Karadag *et al.*, 2019, Marcellin *et al.*, 2019).

Although the impact of endometriosis *per se* on ovarian reserve as measured by AMH levels and oocytes retrieved is clear, its effect of future fertility is less evident. The chance of pregnancy from assisted reproductive technology was not lower in women with bilateral endometriomas (without previous surgery) compared to infertile controls (Reinblatt *et al.*, 2011, Benaglia *et al.*, 2013). And a more recent study has found that the existence of endometriomas alone has no effect on the clinical pregnancy and live birth rates after IVF; however, the presence of deep endometriosis was associated with reduced clinical pregnancy and the live birth rates (Ashrafi *et al.*, 2019).

In contrast, a recent study has found that endometriosis patients with high AMH levels have a significantly higher cumulative pregnancy rate than those patients with low AMH levels, suggesting that pre-treatment AMH levels might be a useful marker to predict the occurrence of natural pregnancy (Zhou *et al.*, 2019). Nevertheless, more studies are needed to confirm the usefulness of ovarian reserve testing in order to support FP decisions in women with endometriosis.

Endometriosis treatment and, specifically, surgical removal of the cysts has also been proven to have an important impact on ovarian reserve and function, with studies showing a decrease in AMH levels and number of oocytes responsive to ovarian stimulation, compromised ovarian function tests, and decrease in age at menopause in women after laparoscopic stripping of ovarian endometriomas (Coccia *et al.*, 2011, Somigliana *et al.*, 2011, Raffi *et al.*, 2012, Somigliana *et al.*, 2012, Turkcuoglu and Melekoglu, 2018). Other techniques, however, may be less detrimental (Zaitoun *et al.*, 2013, Candiani *et al.*, 2018, Sweed *et al.*, 2018). A recent study has found that the long-term effects of endometriomas cystectomy decreasing AMH levels, might be more significant in patients with larger and bilateral cysts, whereas only short-term effects are seen in patients with smaller and unilateral cysts (Wang *et al.*, 2019).

Recommendation

The relevance of ovarian testing to help guide fertility preservation options or treatment decisions in endometriosis patients remains inconclusive.	**WEAK** ⊕○○○

In patients with endometriosis, the involvement of the ovaries and the radicality of surgery influence ovarian reserve as measured by AMH levels, however their effect on future fertility is unclear.	**GPP**

Justification

Patients with severe endometriosis, particularly bilateral endometriomas, are at high risk of POI and lower AMH levels. Surgical treatment can further impact on ovarian reserve and AMH levels. The relevance of pre-treatment AMH levels to predict the chance of future pregnancy or the need for fertility preservation is unclear, as studies reporting on this have made conflicting conclusions.

If AMH levels are measured, the GDG suggests doing so after surgery based on the significant negative impact surgery may have.

Other diseases and interventions

The reproductive function of women has been shown to be affected in many other conditions (Table 8). Substantial evidence from several studies demonstrates that low AMH levels and other biomarkers of ovarian reserve are affected in many diseases (either by the disease itself or due to the gonadotoxic effects of their treatment) or by medical interventions (such as gender reassignment surgery). In these instances, the relevance of ovarian reserve testing for predicting long-term ovarian failure or fertility issues remains inconclusive.

A list of indications in which ovarian reserve testing has been performed is available in Table 8. However, the relevance of the ovarian reserve test to guide decisions of fertility preservation in these diseases remains inconclusive.

Recommendation

For women with overt POI, fertility preservation is not recommended.	**GPP**

For women with reduced ovarian reserve (Bologna criteria, AMH 0.5-1.1ng/ml), advise needs to be individualized and the value of FP is unclear.	**GPP**

205 Table 8 Overview of benign medical diseases for which ovarian reserve testing has been
206 performed

Disorder/Disease	Observations of ovarian reserve tests	Reference
Autoimmune diseases		
Autoimmune thyroid disease	Lower pre-treatment AMH levels than HC	(Magri *et al.*, 2015, Saglam *et al.*, 2015)
Rheumatoid arthritis	Lower pre-treatment AMH levels than HC	(Henes *et al.*, 2015)
Early rheumatoid arthritis	Same AMH levels than HC	(Brouwer *et al.*, 2013)
Juvenile Idiopathic arthritis	Lower AMH levels than HC, AMH levels not related to time to pregnancy	(Ferreira *et al.*, 2019)
Spondyloarthritis	Lower pre-treatment AMH levels than HC	(Henes *et al.*, 2015)
Behçet's disease	Lower pre-treatment AMH levels than HC	(Henes *et al.*, 2015)
	No difference in AMH, AFC, FSH or LH levels with HC	(sahln *et al.*, 2017)
Antiphospholipid syndrome	More patients with low AFC count and AMH levels	(Yamakami *et al.*, 2014)
	Antiphospholipid levels in blood were correlated to AMH levels in infertile women	(Vega *et al.*, 2016)
Takayasu arteritis	More patients with low AFC count and AMH levels	(Mont'Alverne *et al.*, 2015)
Crohn's disease	Lower AMH levels in >30 years old	(Freour *et al.*, 2012)
	Lower AMH levels if disease is restricted to colon	(Freour *et al.*, 2012)
	Lower AMH levels in patients. AMH levels inversely correlate to disease activity index.	(Senates *et al.*, 2013)
IBD patients treated with Thalidomide	Treatment with thalidomide decreases AMH levels and AFC	(Peng *et al.*, 2017)
Granulomatosis with polyangiitis	Treatment with CP decreases AMH levels	(Clowse *et al.*, 2011)
Wegener's syndrome	CP decreases AMH levels in patients	(Clowse *et al.*, 2011)
Multiple sclerosis	Lower AMH, AFC and ovarian volume in high disease activity index patients	(Sepulveda *et al.*, 2016)
	Lower AFC and OV, higher LH in MS treated with immunomodulatory drugs compared to HC	(Cil *et al.*, 2009)
Sjogren's syndrome	Lower AMH, AFC and higher LH in patients compared to HC	(Karakus *et al.*, 2017)
Fragile X and Turner Syndrome		
Fragile X syndrome	Lower AMH, AFC and OV in carriers versus non-carriers	(Tsafrir *et al.*, 2010)
	Lower AMH levels in longer sequence repeats than shorter sequence repeats	(Rohr *et al.*, 2008)
	Higher FSH in carriers	(Welt *et al.*, 2004)
	No correlation between FSH and the number of CGG repeats in fragile X premutation carriers,	(Welt *et al.*, 2004)
Turner Syndrome	AMH correlates to ovarian function	(Hagen *et al.*, 2010)
	AMH levels associate to spontaneous pubertal development	(Hamza *et al.*, 2018)
Other diseases		
Galactosemia	Lower AMH than HC	(Sanders *et al.*, 2009)
	AMH correlates to spontaneous menarche	(Frederick *et al.*, 2018)
Fanconi Anaemia	Lower AMH than HC	(Sklavos *et al.*, 2014)
Sickle cell disease	Lower AMH than HC	(Kopeika *et al.*, 2019)
Beta Thalassemia	Lower AMH and AFC in women with transfusion dependent beta thalassemia than HC	(Talaulikar *et al.*, 2019)
Diabetes I	Lower AMH and Inhibin B, than HC (Specially at later reproductive ages)	(Kim *et al.*, 2016, Wellons *et al.*, 2017)
Bone Marrow Syndrome	Lower AMH than HC	(Sklavos *et al.*, 2015)
Interventions		
Gender reassignment	AMH levels reduced after GnRH and testosterone treatment in gender reassignment	(Caanen *et al.*, 2015)

Abbreviations: AFC, antral follicle count; AMH, anti-Müllerian hormone; CP, cyclophosphamide; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HC, healthy controls; IBD, inflammatory bowel disease; LH, luteinizing hormone; MS, Multiple sclerosis; OV, ovarian volume.

Elective oocyte cryopreservation

Oocyte cryopreservation is an increasingly common method for women to guard against the natural age-related fertility decline (Saumet *et al.*, 2018). Both ovarian reserve and age are the important patient features that determine the ovarian response to stimulation. There is a clear correlation between AFC and serum AMH levels with oocyte yield to stimulation (Nelson *et al.*, 2013, Saumet *et al.*, 2018, Sonigo *et al.*, 2019). Therefore, ovarian reserve testing is commonly used to tailor ovarian stimulation strategies and maximize follicular recruitment if a poor response is anticipated (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020).

However the ability of ovarian testing using AMH levels to predict embryo quality and chances to conceive has not been demonstrated (Dewailly and Laven, 2019, Sonigo *et al.*, 2019). Therefore, ovarian reserve testing should not be measured for making FP decisions.

Recommendation (as in (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020))

For predicting high and low response to ovarian stimulation, use of either antral follicle count (AFC) or anti-Müllerian hormone (AMH) is recommended over other ovarian reserve tests.	**STRONG** ⊕⊕◯◯

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PART D: Fertility preservation interventions

D1. Options for FP

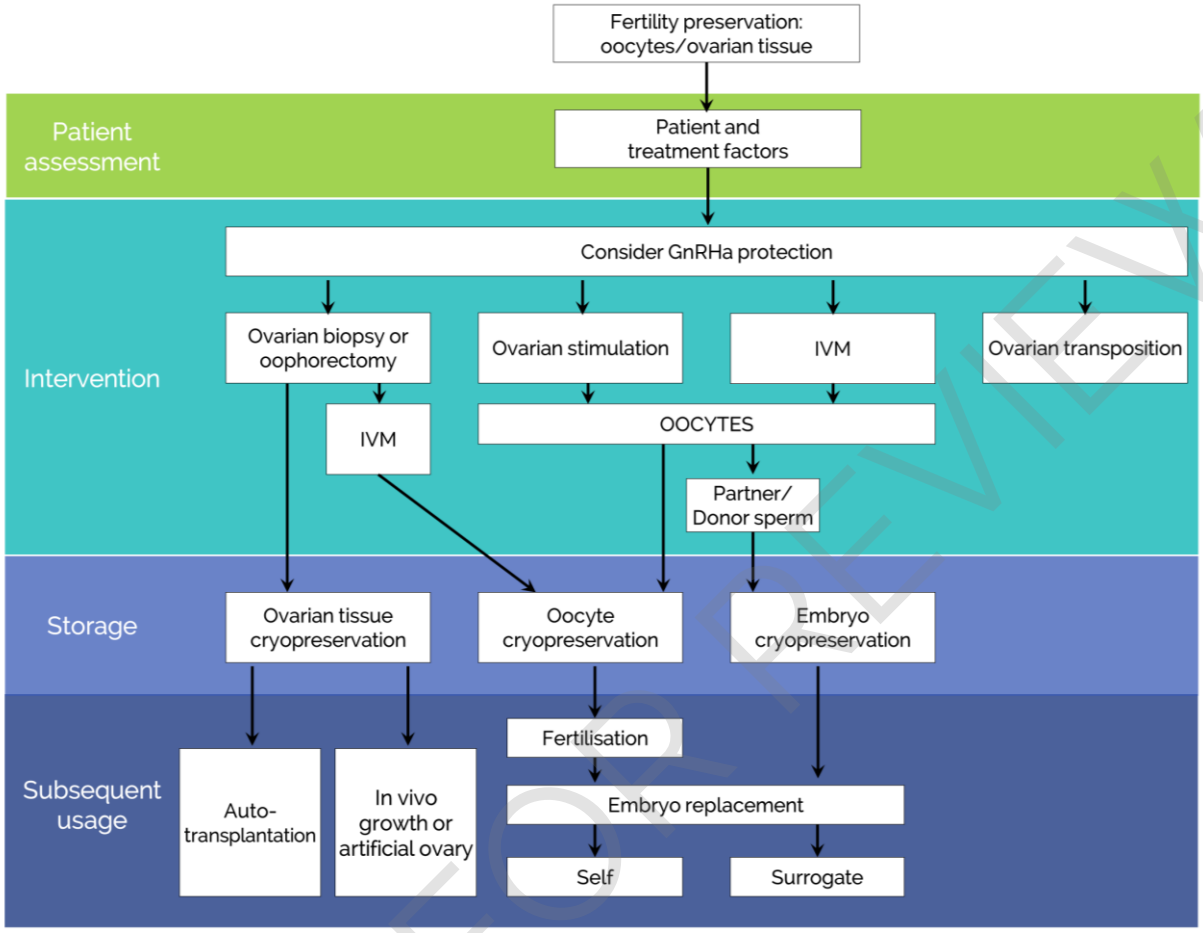
Narrative Question: Which options are available for fertility preservation in women – emergency and non-emergency?

Fertility can be preserved through several procedures, including cryopreservation of oocytes, embryos or ovarian tissue, and potentially medical and surgical methods of protection (see overview Figure 4). Since the development of vitrification, oocyte cryopreservation (section D3. Oocyte cryopreservation) is the method of choice for women undergoing elective freezing, and for most women undergoing fertility preservation for medical indications. Embryo cryopreservation (section D5. Embryo cryopreservation) is even more widely available and long-established part of assisted reproduction, but the necessity for joint legal ownership with the male partner is an important consideration that may result in difficulties later on. Ovarian tissue cryopreservation (section D6. Ovarian tissue cryopreservation) is an important option either through choice, or if there is insufficient time for ovarian stimulation. Its use in prepubertal girls is outwith the remit of this Guideline. In vitro oocyte maturation (section D7 In vitro maturation) can also be considered, and in some cases, there may be a possibility of combining different approaches. The application of these techniques for transgender men is also discussed.

Protection of the ovary against the effects of treatment remains an ideal option, though far from achievable. The use of GnRH agonists (section D8. GnRH agonists) in this regard has a long history, but only recently have more robust data from RCTs become available, and even then, the great majority of the evidence is in women with breast cancer. Ovarian transposition (section D9. Ovarian transposition) in women scheduled for pelvic radiotherapy is also discussed.

[74]

Figure 4 Schematic overview of the options for female fertility preservation. Adapted from (Anderson *et al.*, 2015)



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D2. Ovarian Stimulation in treatments aimed at FP

Oocyte vitrification and embryo cryopreservation are both well-established fertility preservation (FP) methods in widespread clinical practice. Both methods require ovarian stimulation, a clinical procedure widely applied in treatments for infertility and recently discussed in the ESHRE Ovarian Stimulation Guideline (The ESHRE Guideline Group on Ovarian Stimulation et al., 2020). For the purpose of this guideline on Fertility Preservation, only aspects of ovarian stimulation relevant for patients undergoing FP will be discussed.

Ovarian stimulation for FP is usually an urgent procedure and evidence on feasibility, efficacy and safety of the methods is needed. The collection of a sufficient number of oocytes within a limited time frame may be challenging; safety issues include both the prevention of complications such as OHSS, and potentially increased risks relating to the impact of FP in an underlying malign or benign disease. Novel approaches have been suggested for specific cases or patient groups, such as random-start ovarian stimulation, and ovarian stimulation in the context of estrogen-sensitive cancer.

For the current chapter, we include the evidence collected in the recent ESHRE Guideline on Ovarian Stimulation (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020). When considered relevant, additional information was added from studies published after the publication of the Ovarian Stimulation guideline. A discussion of recommendations that were updated is also presented.

PICO Question: how should ovarian stimulation be performed in cancer patients undergoing FP treatment?

Preferred protocol

Evidence as in the ESHRE Guideline on Ovarian stimulation (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020) (section 10.1)

Two systematic reviews including a total of 33 studies (Boots *et al.*, 2016, Rodgers *et al.*, 2017) and 14 other investigations (Lawrenz *et al.*, 2010, Lee *et al.*, 2010, Das *et al.*, 2011, Garcia-Velasco *et al.*, 2013, Johnson *et al.*, 2013, Devesa *et al.*, 2014, Cardozo *et al.*, 2015, Chan *et al.*, 2015, Shapira *et al.*, 2015, Druckenmiller *et al.*, 2016, Pereira *et al.*, 2016, Alvarez and Ramanathan, 2018, Muteshi *et al.*, 2018) reported data on cancer patients having ovarian stimulation for oocyte and/or embryo cryopreservation. More than 2200 cycles were described, most of them (>90%) with GnRH antagonist protocols. Among them, random-start ovarian stimulation or protocols were included, as well as the use of aromatase inhibitors or tamoxifen in women with breast cancer. In addition, different trigger types aiming at the final oocyte maturation were used. The main outcome measure across studies was usually the overall number of oocytes recovered and the number of mature oocytes obtained, as data on embryo replacement and live birth are scarce.

Evidence (published since (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020))

Subsequently to the publication of the ESHRE Guideline on ovarian stimulation, a prospective study of fertility preservation in women with breast cancer reported on 380 cycles using a GnRH antagonist regimen. The use of letrozole or random start (each in approximately half of all cycles) was not associated with differences in the number of oocytes or embryos cryopreserved compared to conventional approaches (Marklund, 2020).

Recommendation

[76]

For ovarian stimulation in women seeking fertility preservation for medical reasons the GnRH antagonist protocol is recommended for its feasibility in urgent situations, short time and safety reasons.	**STRONG** ⊕○○○

For patients requiring ovarian stimulation where there is a lack of urgency, the use of a long protocol may be appropriate.	**WEAK** ⊕○○○

Justification

The GnRH antagonist protocol has advantages due to a shortened duration of stimulation and allowing triggering of oocyte maturation with a GnRH agonist in high responder women, which further reduces the risk of OHSS. The GDG judged that a strong recommendation for the use of protocols with GnRH antagonists would be appropriate for emergency FP, especially with regards to safety reasons and time constraints and this was added as such in the recommendation. For non-urgent ovarian stimulation, the planning of cycles using GnRH agonist protocols is feasible and could be used if preferred, as a good practice point (GPP).

Random-start protocol

Evidence as in the ESHRE Guideline on Ovarian stimulation (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020) (section 10.2)

A systematic review of 8 non-randomized studies including 6 in a context of fertility preservation, showed in 251 women, that ovarian stimulation cycles initiated in the luteal were slightly longer (Weighted Mean Differences (WMD) 1.3 days, 95% CI 0.37–2.1) and required higher gonadotropin doses (WMD 683 IU, 95% CI 369–997), when compared with stimulation started in the follicular phase (Boots *et al.*, 2016). Peak serum estradiol (WMD −337 pg/mL, 95% CI −849 to −175) and number of oocytes recovered (WMD −0.6 oocytes, 95% CI −2.8 to 1.6) did not differ between phases of the cycle at which OS was started. Oocytes obtained in cycles initiated in the luteal phase fertilized more efficiently (WMD 0.16, 95% CI 0.13 to 0.19). No conclusion can be drawn on pregnancy and live birth rates regarding the very small number of patients and the extremely low utilization rates of cryopreserved oocytes and embryos in cancer patients (Boots *et al.*, 2016).

Two retrospective cohort studies, including 347 cancer patients undergoing ovarian stimulation for FP, also compared conventional vs random-start ovarian stimulation (Pereira *et al.*, 2016, Muteshi *et al.*, 2018). Muteshi et al. reported no significant differences in number of oocytes retrieved (11.9 (95% CI 10.3–13.5) vs. 12.9 (95% CI 9.6–16.2)), total gonadotropin dose used (mean 2543.4 (2328.3–2758.5) vs. 2811.9 (2090.8–3533.1) IU), total duration of stimulation (11.5 (11.2–12.0) vs. 12.2 (10.7–13.7) days) or peak serum estradiol (5426.3 (4682.9–6169.7) vs. 4423.1 (2866.9–5979.3) pmol/L) (Muteshi *et al.*, 2018). Similarly, Pereira et al. reported no significant difference in number of oocytes retrieved (12.1±5.78 vs. (12.6±6.23); OR 1.05, 95% CI 0.45–2.45), total gonadotropin dose used (3498.3±1563.1 vs. 3527.4±1668.9 IU), or peak serum estradiol (473.3 (262.4-615.7) vs. 443.8 (285.2-603.5) pg/ml)(Pereira *et al.*, 2016). However, duration of stimulation was significantly longer when ovarian stimulation was started in the luteal phase compared to the follicular phase (11.8 (±2.41) vs. 10.7 (±2.71) days) (Pereira *et al.*, 2016).

Evidence (published since (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020))

In a prospective cohort study of 26 women with cancer, the outcome of 13 FP cycles initiated in the follicular phase was compared with 13 cycles started in the luteal phase. No significant differences were observed regarding to numbers of oocytes collected,maturity rate, nor gonadotropin dose or days of stimulation (Campos *et al.*, 2018). In a larger cohort of 109 women with breast cancer, Cavagna et al., reported outcomes of random-start protocols in early follicular phase (n=41), late follicular phase (n=21), and luteal phase (n=47). Similar numbers of oocytes retrieved, and maturity

rates were reported, but a significant higher FSH or hMG dose were required in the cycles initiated in the luteal phase (Cavagna *et al.*, 2018).

A prospective study compared random start ovarian stimulation in 201 cycles with 179 cases of conventional start in women with breast cancer. Random-start required higher total gonadotropin dose, but the number of retrieved oocytes and the number of cryopreserved oocytes (9.0 [range 0-24] vs 10.6 [range 0-40]) and embryos (4.8 [range 0-29] vs 4.8 [range 0-16]) were similar between the groups (Marklund, 2020).

Recommendation

In urgent fertility preservation cycles, random-start ovarian stimulation is an important option	**WEAK** ⊕⊕◯◯

Justification

While the evidence indicates that oocyte competence is probably not impacted when ovarian stimulation is started in the luteal phase compared to the follicular phase, there are insufficient data on live birth rates to allow conclusions as to its role in ovarian stimulation for fertility preservation. The quality of evidence is still low given the few studies available. The drug marketing approval for gonadotropin use in luteal phase needs to be considered.

Double stimulation

Evidence as in the ESHRE Guideline on Ovarian stimulation (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020) (section 9.3)

Double stimulation, also called "dual stimulation", "duostim" (Vaiarelli *et al.*, 2018) or the "Shanghai protocol" (Kuang *et al.*, 2014), is used experimentally in poor responder patients or cases for urgent fertility preservation. It involves 2 stimulation protocols within the same menstrual cycle: the first starting in the follicular phase, then second immediately after the oocyte pick up, in the luteal phase of the same cycle. Two oocyte pick-ups are therefore performed approximately 2 weeks apart, thus theoretically allows recovery of more oocytes in a shorter time period. As shown in luteal phase stimulation protocols, the quality of oocytes retrieved in the second stimulation appears to be as good as those retrieved in the first stimulation (same euploid embryo rate) (Vaiarelli *et al.*, 2018). Since there are no studies performing the direct comparison of double stimulation with 2 consecutive conventional stimulations, there are no relevant data to present in this guideline. However current evidence shows that double stimulation is feasible and provides oocytes with sufficient quality for IVF/ICSI. The advantages/disadvantages of double stimulation compared to conventional stimulation need to be addressed in randomised controlled studies.

Evidence (published since (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020))

A study in women with poor ovarian response (defined according to the Bologna criteria with mean age 42 years) investigated dual stimulation with PGT-A. In the study, 100 patients, undergoing dual stimulation, were compared to 197 that underwent a single conventional cycle. The cumulative LBR was higher (15% vs 7%) as was the proportion of euploid blastocysts (31% vs 14%) in the group that underwent dual stimulation (Vaiarelli *et al.*, 2020). The high age of the women included and the lack of randomization limit generalisability of these data to FP patients.

Recommendation

Double stimulation can be considered for urgent fertility preservation cycles	**WEAK** ⊕⊕◯◯

Justification

Although not recommended in poor responders (except in the context of a clinical trial), the recent guideline on ovarian stimulation (The ESHRE Guideline Group on Ovarian Stimulation et al., 2020), suggested that double stimulation can be considered in urgent FP cycles. This is based on studies that have reported more oocytes with double stimulation compared to follicular phase stimulation and comparable pregnancy rates from oocytes obtained in the luteal or follicular phase. The disadvantage of mandatory freeze-all of oocytes or embryos resulting from luteal start stimulation is irrelevant in the context of FP.

Ovarian stimulation with potentially safer protocols aiming at reducing estrogenic effects and risks

Fertility preservation in breast cancer represents a complex issue since the tumors are in many cases estrogen-sensitive. Ovarian stimulation results in supra-physiological serum estradiol levels, albeit temporary, which could theoretically result in the proliferation of malignant cells, although there are no data demonstrating an adverse effect of ovarian stimulation for FP in women with breast cancer.

Innovative stimulation protocols have been developed in an effort to reduce potential harm associated with high estradiol levels. Co-administration of either aromatase inhibitors or selective estrogen receptor modulators during ovarian stimulation is used frequently.

Evidence as in the ESHRE Guideline on Ovarian stimulation (**The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020**) (section 10.3)

A systematic review analysed the results of 12 prospective and retrospective cohort studies with aromatase inhibitor protocols for fertility preservation (Rodgers *et al.*, 2017). Peak estradiol concentrations were 337-829 pg/mL (1237-3044 pmol/L) when letrozole was commenced on Day 2-3, higher than that observed in natural cycle IVF. Two studies reported no difference in oocyte yield between aromatase inhibitor protocols and conventional stimulation (Oktay *et al.*, 2006, Checa Vizcaino *et al.*, 2012) while 2 others observed a small but significant decrease with letrozole administration (Domingo *et al.*, 2012, Revelli *et al.*, 2013). However, the amount of FSH administration in Revelli's study was lower in the aromatase inhibitor group, which may have biased the results.

Rodgers et al. also reviewed the 4 prospective and retrospective cohort studies having used tamoxifen administration during ovarian stimulation (Rodgers *et al.*, 2017). Peak estradiol levels in women stimulated with tamoxifen co-administration were higher than observed in natural cycle IVF (Oktay *et al.*, 2003), however, remained comparable in women undergoing ovarian stimulation without tamoxifen (Meirow *et al.*, 2014). One study in the systematic review compared ovarian stimulation with letrozole to that with tamoxifen (Oktay *et al.*, 2005). The numbers of oocytes retrieved of mature oocytes were lower when stimulation was performed with tamoxifen than with letrozole (6.9±1.1 vs. 12.3±2.5) and (5.1±1.1 vs. 8.5±2.6), respectively. However, the small number of patients included (7 women and 9 cycles in the tamoxifen group and 11 women with 11 cycles in letrozole group) means that making conclusions should be cautious.

A retrospective cohort study including 639 women compared ovarian stimulation with letrozole in breast cancer patients versus ovarian stimulatiion without letrozole in women presenting for elective cryopreservation (Pereira *et al.*, 2016). There was no significant difference in the duration of stimulation (10.9±3.46 vs. 10.4±3.69 days), total amount of gonadotropins administered (3502.4±1372.1 vs. 3607.8±1848.6 IU). However, peak serum estradiol was significantly lower in women receiving letrozole (464.5 (315.5-673.8) vs. 1696 (1058-2393) pg/ml). Furthermore, significantly more oocytes were retrieved in women receiving letrozole (12.3±3.99 vs. 10.9±3.86) (Pereira *et al.*, 2016).

The use of GnRH agonist trigger to an antagonist protocol with addition of aromatase inhibitor protocols contributes to further reducing estradiol levels around the time of OPU (Oktay *et al.*, 2010, Reddy *et al.*, 2014). and progesterone levels during the luteal phase (Goldrat *et al.*, 2015).

Data on relapse-free survival and mortality were available in only 4 studies of the systematic review, encompassing 464 women with a maximum of 5-year follow-up.

Evidence (published since (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020))

In a prospective study, cycles including letrozole for FP in women with breast cancer resulted in a similar number of oocytes (10.4 versus 9.1) and embryos (5.5 vs 3.0) cryopreserved compared with cycles with a conventional antagonist protocol (Marklund, 2020). The safety of ovarian stimulation in women with breast cancer was also investigated with median follow up of 5.1 years (range: 3 months-23,6 years). Comparing women who underwent FP with ovarian stimulation to women who did not undergo FP or had FP treatment without ovarian stimulation, the five-year survival was 0.95 (95% CI:0.92-0.97) and 0.92 (95% CI:0.87-0.95), respectively, with no difference in survival across the entire follow-up. Letrozole treatment was also not associated with differences in survival, thus no benefit has been established. A study aiming to compare the short- and long-term effects of ovarian stimulation with or without letrozole co-administration is currently ongoing (STIM-Trial, The Netherlands).

Recommendation

In ovarian stimulation for fertility preservation in estrogen-sensitive diseases the concomitant use of anti-estrogen therapy, such as letrozole, is probably recommended	GPP

Justification

The existing literature concerning ovarian stimulation for FP in women with estrogen sensitive cancer is limited by its observational nature, small patient numbers and relatively short duration of follow-up. Definitive statements regarding the safety of ovarian stimulation in women with a recent diagnosis of breast cancer would require long-term and large-scale studies, and these do not yet exist. The data on use of tamoxifen for FP of women with breast cancer are even more limited than data on letrozole. The FP GDG decided that tamoxifen should not be included in the recommendation.

Ovarian stimulation for FP in transgender men[4]

The procedures required for FP aiming at oocyte cryopreservation, such as hormonal ovarian stimulation and transvaginal ultrasound (TVS), can have a negative impact on gender dysphoria. Successful management requires sensitivity and awareness of these issues (Armuand *et al.*, 2017).

It is undoubtedly preferable for transgender males to undergo procedures for FP aimed at store oocytes before starting gender-affirming hormone treatment (GAHT). In some cases, the patients may agree to temporary discontinue their GATH to undergo ovarian stimulation aiming at oocyte vitrification. The use of long-term testosterone treatment, in certain cases with treatment with a GnRHa, may result in the patients being severely downregulated and hypogonadotrophic, comparable to women on long-term GnRHa treatment for endometriosis.

Discontinuation of testosterone treatment prior to ovarian stimulation for FP in transgender men has been reported, using antagonist protocols (Adeleye *et al.*, 2019, Leung *et al.*, 2019). Seven transgender men who had discontinued treatment with testosterone were compared with 6 transgender men without previous treatment with testosterone. Time from stopping testosterone was not reported. Fewer oocytes were retrieved in patients with previous testosterone use (12 IQR [4-26]) vs. 25.5 [18-28]) (Adeleye *et al.*, 2019). In another report, 19 transgender men underwent cycles for oocyte or embryo cryopreservation, and 7 underwent cycles with embryos transferred

[4] this topic was not included in The ESHRE Guideline Group on Ovarian Stimulation, Bosch E, Broer S, Griesinger G, Grynberg M, Humaidan P, Kolibianakis6 E, Kunicki M, La Marca A, Lainas G *et al.* ESHRE guideline: ovarian stimulation for IVF/ICSI. *Human reproduction open* 2020: and www.eshre.eu/guidelines.

(Leung *et al.*, 2019). Over 60% of the patients had been on treatment with testosterone (range 3 months – 17 years). All patients stopped testosterone before cycle start (average 4 months, range 1-12 months) and almost all resumed resumption of menses and had normal baseline FSH, AMH and E2 levels at cycle start. A similar number of oocytes were retrieved and peak E2 levels were found compared to cisgender women undergoing treatment for infertility (Leung *et al.*, 2019).

The addition of aromatase inhibitors has been proposed to further reduce systemic estrogen levels and estrogenic symptoms (Armuand *et al.*, 2017).

Recommendation

For ovarian stimulation in transgender men aiming at oocyte cryopreservation, GnRH antagonist protocols have been reported as feasible and with numbers of oocytes retrieved comparable to those obtained in cisgender women when individuals have stopped previous treatment with testosterone	**WEAK** ⊕○○○

Addition of letrozole to the antagonist protocol may enhance treatment adherence for transgender men by reducing estrogenic symptoms.	**GPP**

Justification

Published data on ovarian stimulation in transgender men are limited to small case series, but show feasibility of ovarian stimulation, even in patients that have previously used testosterone treatments. Ovarian stimulation can impact negatively on gender dysphoria, and hence sensitivity and awareness, and protocal adaptation can be considered.

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D3. Oocyte cryopreservation

Cryopreservation of mature oocytes through vitrification has shown proof of its efficacy in egg banking programs and in elective oocyte cryopreservation. These facts contributed to an international consensus in 2013 to recognize oocyte cryopreservation as a clinically established method for female fertility preservation (Loren *et al.*, 2013, 2013, Yasmin *et al.*, 2018). Although large studies on oocyte cryopreservation are available, most still report on healthy women undergoing elective oocyte cryopreservation or on oocytes used in donor cycles. The number of women who have returned to use frozen oocytes after FP indicated for malignant or benign medical indications is still low

PICO Question: Is oocyte cryopreservation effective and safe for fertility preservation?

Several studies have confirmed the feasibility of oocyte cryopreservation for FP of adult women and young teenagers (Rudick *et al.*, 2010, Rienzi *et al.*, 2012, Druckenmiller *et al.*, 2016, Mangili *et al.*, 2017, Rodriguez-Wallberg *et al.*, 2019b) and the reported number of oocytes collected among studies is similar. Druckenmiller et al. reported an average of 10 mature oocytes collected per cycle in a cohort of 176 women with cancer undergoing 182 cryopreservation cycles (Druckenmiller *et al.*, 2016). Mangli et al also reported a retrospective analysis of 125 women with cancer undergoing FP in two different study periods and found that a mean of 8 and 10 oocytes were cryopreserved, respectively (Mangili *et al.*, 2017). In a prospective study, Rodriguez-Wallberg et al. reported on 180 women with benign diseases and 382 women with malignant diseases and found a mean of 12 oocytes retrieved in each of the groups(Rodriguez-Wallberg *et al.*, 2019a). However, there was a significantly higher number of mature oocytes obtained in the group of patients with benign versus malignant indications, resulting in greater numbers of mature oocytes cryopreserved for women with benign indications (12.3 ± 7.1 vs. 9.8 ± 6.9) (Rodriguez-Wallberg *et al.*, 2019a).

A large study conducted in the USA during 2009 reported data collected from 282 centers. The oocyte cryopreservation cycles were indicated by cancer in 18% of cases whereas 66% were elective. Fertilization rates after warming were about 67%, and 337 live births from 857 warming cycles were reported for all indications combined, with a pregnancy rate of 39.3% (Rudick *et al.*, 2010).

Effect of age and/or previous cancer treatment

It can be assumed, as in infertile patients or women undergoing elective oocyte cryopreservation, that in women undergoing FP, increasing age at time of cryopreservation has a negative effect on the outcome of oocyte cryopreservation. Furthermore, it was shown that women who are older require higher gonadotropin doses for ovarian stimulation, as well as women who undergo cryopreservation cycles after chemotherapy treatment (Rodriguez-Wallberg *et al.*, 2019a).

Effect of type of malignancy

It has been difficult to establish if there are patient groups that are disadvantaged as regards to the ovarian response to ovarian stimulation due to their oncologic disease. Studies of large size are lacking. In a retrospective study comparing ovarian stimulation outcomes of 191 women with breast cancer vs 398 women undergoing elective oocyte cryopreservation, Quinn et al. reported a similar number of mature oocytes collected in analysis adjusted for age, BMI and total gonadotropin dose (Quinn *et al.*, 2017). In another retrospective study, data on 306 women who had ovarian stimulation for FP for several indications were analyzed (Lekovich *et al.*, 2016). The most common diseases were breast cancer (n=145, 47.4%), haematological malignancies (n=42, 13.7%), gynaecological (n=20,

6.5%) and gastrointestinal cancer (n=20, 6.5%). Patients with haematological malignancies had more mature oocytes retrieved, while patients with gynaecological malignancy had smaller numbers of oocytes retrieved. These data are in contrast to a study which found that patients with lymphoma had lower AMH levels, and had significantly fewer oocytes retrieved and vitrified after ovarian stimulation (8.1±5.5 versus 9.6±6.4) than women with other malignancies (Lekovich *et al.*, 2016). Recent studies have not found any conclusive data to indicate an effect of the type of cancer in the outcome of ovarian stimulation aimed at FP (Lefebvre *et al.*, 2018). In women with breast cancer, carriers of a BRCA mutation have also presented with similar ovarian reserve and response to stimulation as noncarriers (Gunnala *et al.*, 2019).

Oocyte cryopreservation in adolescents

The feasibility of ovarian stimulation and oocyte cryopreservation in adolescent girls has been reported from several centers that have large programs for FP (Rodriguez-Wallberg *et al.*, 2019a, Manuel *et al.*, 2020), Using the database of the Society for Assisted Reproductive Technology (SART) Clinic Outcome Reporting System (SART CORS) in the USA, cycles aimed at oocyte cryopreservation in adolescents younger than 20 years of age accounted for 1.5% of all oocyte cryopreservation cycles between 2007-2018 (Hipp *et al.*, 2019).

Efficacy of cryopreserved oocytes for fertility preservation

Although a large number of reports are available on oocyte cryopreservation, studies reporting on the efficacy of cryopreserved oocytes are substantially fewer and smaller than those reporting on the use of fresh oocytes. This is even more the case for women who have used oocytes cryopreserved for FP for medical indications. In the study of Druckenmiller et al, only 10 of 176 women returned to thaw their oocytes, and embryos for transfer were obtained in 9 of 11 cycles (Druckenmiller *et al.*, 2016). The implantation rate was 27% and the LBR was 44% (95% CI 12-77%) per ET (Druckenmiller *et al.*, 2016). A larger retrospective multicentric study from Cobo et al. reported on 1073 women who underwent oocyte cryopreservation indicated by an oncologic disease and 5289 healthy women who attempted elective oocyte cryopreservation(Cobo *et al.*, 2018). Both age and indication for oocyte cryopreservation were found to have a marked impact on the cumulative live birth rate (CLBR). Eighty women with previous oncologic indication and 641 from the elective group attempted pregnancy, resulting in CLBR of 41.1 vs 68.8%, respectively. Increasing age from 36 years onwards was associated with lower CLBR. In the group of women younger than 36 years, differences such a lower oocyte survival, fewer embryos obtained and transferred and lower PR and CLBR were found in women with an oncologic indication for FP compared to healthy women who underwent elective oocyte cryopreservation (Cobo *et al.*, 2018).

Effect of oncologic disease vs non-oncologic disease in reproductive outcome of oocyte vitrification cycles

A prospective study of 562 adult women who had undergone oocyte or embryo cryopreservation for medical indications found a similar return rate of 27% regardless of benign or malignant indication (Rodriguez-Wallberg *et al.*, 2019a). A significantly lower CLBR was found after warming cycles in women with oncologic versus benign indications (LBR 21% vs 47%)(Rodriguez-Wallberg *et al.*, 2019a).

Effect of type of cancer diagnosis on outcome of oocyte cryopreservation

In the study of Lekovich et al , including 306 women undergoing FP for several malignant indications, fertilization rate and the number of cancelled cycles were comparable among all diagnosis groups. Thirty-two embryo transfer cycles in 22 patients resulted in a PR per ET of 43.75%, and cumulative PR per patient 54.5%. Live birth rate per patient was 22.72% (Lekovich *et al.*, 2016) .

Safety and risks

In studies of FP for cancer patients, a period of about 2-weeks has been needed in general to obtain oocytes (Druckenmiller *et al.*, 2016, Mangili *et al.*, 2017, Rodriguez-Wallberg *et al.*, 2019a). That seems to be an acceptable time span between diagnosis and initiation of cancer therapy in most cases (Loren *et al.*, 2013).

General risks of ovarian stimulation and oocyte pick-up

Fertility preservation cycles should be considered only in women with no obvious contraindication for ovarian stimulation and/or oocyte pick-up.

Specific risks of ovarian stimulation for fertility preservation in women with cancer or benign diseases may be related to the altered endocrine environment, and risks for thrombosis, haemorrhage and infection should be considered in all cases. In women with estrogen-sensitive cancer, the potentially deleterious role of supra-physiological estradiol levels during ovarian stimulation may be reduced by the addition of aromatase inhibitors alongside gonadotropin stimulation (Oktay *et al.*, 2018). The risks of thrombotic complications may be increased in women with certain diseases including malignant conditions in general, and autoimmune or rare diseases, as reported in women with GATA2 deficiency (Zolton *et al.*, 2018).

Patients suffering from diseases featuring low platelet counts or lymphopenia may present with inherent higher risks of bleeding and/or infection following transvaginal puncture procedures for oocyte pick-up.

In all patients, the potential risk of OHSS should be considered, in particular if they are young or expected to be high responders. OHSS should be avoided in women undergoing FP for medical reasons due to theoretically increased risks of complications such as thrombosis, in addition to potentially delaying a planned cancer treatment. It has been established in large studies that the risk of OHSS increases when >15 oocytes are collected (Steward *et al.*, 2014, The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020). No increased risks have been reported in women with either benign or malignant indications undergoing ovarian stimulation aiming at cryopreservation cycles when a mean of 10-12 oocytes have been retrieved (Mangili *et al.*, 2017, Rodriguez-Wallberg *et al.*, 2019a) although case series of sufficient size to give accurate risk estimates are missing, and will require multicentric international data collection.

Use of aromatase inhibitors for FP in women with hormone-sensitive cancer

This topic is discussed in more detail in section D2. Ovarian Stimulation . Protocols using letrozole have been specifically recommended for women with hormone-sensitive tumours such as estrogen receptor (ER)-positive breast cancer undergoing ovarian stimulation for fertility preservation (Loren *et al.*, 2013). Prospective studies with long-term follow-up of women with breast cancer that have undergone ovarian stimulation for fertility preservation are reassuring and no increased risk of relapse has been found (Azim *et al.*, 2008, Rodriguez-Wallberg *et al.*, 2018). Cycles using letrozole may be also potentially safer for women with endometrial hyperplasia or borderline ovarian tumours (Mangili *et al.*, 2017). The addition of letrozole to ovarian stimulation has also been proposed for patient groups where systemic estradiol increase is not desirable, such as transgender men,to reduce estrogenic effects and the worsening of gender dysphoria (Armuand *et al.*, 2017) and the case of patients with increased inherent thrombosis risk (Zolton *et al.*, 2018). A further improvement proposed to further minimize the risk of OHSS with letrozole is the use of GnRH agonist for oocyte trigger instead of hCG (Oktay *et al.*, 2010, Goldrat *et al.*, 2015).

The use of letrozole in cycles for fertility preservation, as well as within fertility treatments, is widely accepted, however, still off-label.

Potential risks to offspring associated with oocyte cryopreservation

Observational data indicate that children conceived using cryopreserved oocytes do not have an increased risk of congenital anomalies, but the data are too limited for definitive analysis. A review of 936 live-born babies from 58 cryopreservation studies 1986-2008 indicated an incidence of 1.3%

of congenital anomalies, a rate comparable to the 3% rate of major structural or genetic birth defects found in live births in the USA (Noyes *et al.*, 2009). Long-term cryopreservation does not increase embryonic aneuploidy when compared to fresh oocytes (Goldman *et al.*, 2015). Studies with long-term follow up of children are lacking. While children conceived from assisted reproduction have an elevated risk of adverse birth outcomes (Goisis *et al.*, 2019), it is likely that the increased risks are related to the subfertility of the couple; there are currently insufficient data to assess these risks after FP.

Choice of cryopreservation of oocytes versus embryos.

Cryopreserved oocytes will always belong to the woman. If a couple is being treated, resulting in embryo storage, the embryos belong to the couple. If the couple separates in the future, or if the man does not consent to using the embryos, the woman is not be able to use the embryos for attempting pregnancy. A recent prospective study of FP investigating trends in patients' choices found that more than half of the women with a partner chose either not to fertilize their oocytes aiming at cryopreservation of oocytes only or to share obtained oocytes attempting both cryopreservation of oocytes and cryopreservation of embryos (Rodriguez-Wallberg *et al.*, 2019a). Women should receive information on the relevant legal issues and should have the possibility to elect to cryopreserve embryos or oocytes, or to split the oocytes aiming at both methods. More accurate data on CLBR after oocyte and embryo vitrification for FP for medical indications would also be of value to inform patients who have a choice as to what to cryopreserve.

Recommendations

Oocyte cryopreservation should be offered as an established option for fertility preservation.	**STRONG** ⊕⊕◯◯
Women with a partner should be offered the option to cryopreserve unfertilized oocytes or to split the oocytes to attempt both embryo and oocyte cryopreservation.	**GPP**
Women should be informed of accurate, centre-specific expertise and live birth rates. They should also be informed that success rates after cryopreservation of oocytes at the time of a cancer diagnosis may be lower than in women without cancer.	**GPP**

Justification.

The majority of studies are retrospective (Cobo et al., 2013, Druckenmiller et al., 2016, Massarotti et al., 2017, Cobo et al., 2018) and only a few prospective studies are available on women undergoing cycles for FP due to malignant diseases or benign conditions (Rienzi et al., 2012, Rodriguez-Wallberg et al., 2019a). Overall, evidence suggests that oocyte cryopreservation is effective and safe for patients undergoing FP, even though long-term follow-up of the children born after treatment are not available. It is expected that the number of publications on the outcome of warming cycles in these patients will increase in the coming years. Evidence on safety and efficacy of ovarian stimulation and oocyte pick-up, as necessary preceding steps to oocyte cryopreservation, as summarized in the previous section, is also considered in this recommendation.

For women without a partner, oocyte cryopreservation is probably the most straightforward option, but also for women with a partner, this is probably appropriate. Embryo cryopreservation as the alternative can be associated with possible ethical and legal consequences. Patients should receive information on both choices and elect their preferred option. There may be specific situations where the use of donor sperm and embryo cryopreservation can be considered, for instance genetic siblings. Furthermore, local legislation should be considered.

The GDG decided that this recommendation for information provision is necessary and defendable.

Research recommendation

Studies reporting on birth outcomes, prevalence of genetic syndromes and long-term follow-up of children conceived using cryopreserved oocytes are needed in order to assess the overall safety of oocyte cryopreservation.

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D4. Oocyte cryopreservation for non-medical reasons

Elective oocyte cryopreservation or egg freezing, i.e. choosing to cryopreserve their oocytes with no medical indication, is increasing and has largely replaced embryo cryopreservation as a fertility preservation option for women without a male partner (see also section on embryo cryopreservation). Women opting for such elective preservation are usually young and healthy, they do not, generally, have pre-existing medical problems. Therefore, the clinical issues are arguably more straightforward for this group. The motivations are different however, as they are not freezing to ameliorate a medical condition but for other, often complex reasons. Women in the developed world are delaying the age at which they have their first child . The reasons for this demographic trend have been extensively debated, with several cultural and socio-economic reasons advanced (Baldwin *et al.*, 2018).

Current guidelines on elective oocyte cryopreservation

International guidelines generally support elective oocyte cryopreservation as a technique but recommend that it should be used with caution. The ESHRE Ethics taskforce paper on oocyte cryopreservation for age related fertility loss states: 'It is concluded that the arguments against allowing this application of the technology are not convincing' (Dondorp *et al.*, 2012).' However, they stress the need, 'for adequate information of women interested in oocyte cryopreservation, to avoid raising false hopes. The message is that a women's best chances of having a healthy child are through natural reproduction at a relative early age.' Recent guidance from the American Society for Reproductive Medicine (ASRM) states: 'The Committee concludes that planned oocyte cryopreservation may allow women who, in earlier times, would have faced infertility and childlessness to potentially have a child to whom they are genetically linked. Planned oocyte cryopreservation is an ethically permissible medical treatment that may enhance women's reproductive autonomy and promote social equality' (Ethics Committee of the American Society for Reproductive Medicine, 2018). A recent RCOG Scientific Opinion piece on the topic stated that elective oocyte cryopreservation provided an opportunity for women to mitigate the decline in their fertility with age, but highlighted that women undertaking oocyte cryopreservation should only do so with a full understanding of the likelihood of success, as well as costs and risks (Anderson *et al.*, 2020).

Debates over elective oocyte cryopreservation

Elective oocyte cryopreservation has caused some controversy and the ethical acceptability of the practice has been discussed. It can be seen as a useful procedure that can extend women's fertility options and in doing so enhance the individual's reproductive autonomy. The level of evidence of harm needed to justify restricting reproductive choices should be higher than the level needed to justify the restriction of less important choices. Further, reproductive choices are a very important, central aspect of peoples' lives and allowing people to exercise them is a good in itself (Jackson, 2006).

It has been argued that non-medical oocyte cryopreservation can alleviate the gender inequality created by women and men having different age-related biological fertility decline, by allowing women to extend their reproductive years. In their summary of the arguments for elective oocyte cryopreservation the ASRM state: 'Planned oocyte cryopreservation may also promote social justice by reducing the obstacles women currently face because their reproductive window is smaller than men's.' The cost of elective oocyte cryopreservation may conversely increase social inequality as it is only available to women who can afford the significant financial outlay.

It is possible that oocyte cryopreservation could be better for any future child, as this technology gives people more time to prepare, become financially secure, and women will not rush into reproducing when they are not ready or they have not met the 'right' partner (Goold and Savulescu, 2009). It could reduce the incidence of aneuploidy associated with older motherhood.

As oocyte cryopreservation is acceptable for women who have iatrogenic fertility loss i.e. due to cancer treatment, or other medical fertility problems then, arguably, there are no good reasons for making a distinction between these two groups and any unequal treatment is unfair (Dondorp and De Wert, 2009).

There have been a number of objections to elective oocyte cryopreservation. A key objection is that it further medicalises reproduction and could lead to greater commercialisation of reproduction, with the use of inappropriate high-pressure sales practices. However, both ESHRE and ASRM have concluded that elective oocyte cryopreservation does not produce substantial harms and therefore there are no convincing arguments (with regards to harms) to restrict its use. The RCOG highlighted that women undertaking oocyte cryopreservation should only do so with a full understanding of the likelihood of success, as well as costs and risks (Anderson *et al.*, 2020). It also highlighted the need for better education of men as well as women on the impact of age-related changes in female fertility.

Issues with elective oocyte cryopreservation

There are a number of issues that need to be considered when offering elective oocyte cryopreservation services:

- **Success rates,** i.e. the likelihood of achieving a pregnancy after thawing and the effects of the woman's age when using the oocytes; Elective oocyte cryopreservation had a very high cumulative live birth rate (CLBR) for those who froze before they were 35 years old approaching 95% provided sufficient oocytes were obtained (with a 43% CLBR from 10 oocytes) (Cobo *et al.*, 2018). However, a maximum CLBR of 50% was achieved by those who froze when they were over 35 (with CLBR of 25% with 10 oocytes frozen). Thus, age at cryopreservation is key and patients should be made aware of this.
- **Likelihood of using the oocytes;** There are limited data on this (Alteri *et al.*, 2019). Cobo et al (2018) reported that 12.1% of women returned to use their oocytes, with a mean storage time of 2.1 years (Cobo *et al.*, 2018). Stoop et al (2015) found that 29.2% of women indicated that they currently consider the use of frozen oocytes less likely than anticipated at time of oocyte pick up (Stoop *et al.*, 2015).
- **Medical harms**; Oocyte pick-up is not without risks to the woman. These risks will be low as women electively cryopreserving oocytes are likely to be healthy.
- **Obstetric risks;** Importantly, there are risks of delaying childbirth due to older age at time of pregnancy. These risks particularly increase after the age of 45 (Aoyama *et al.*, 2019).
- **Long-term data;** Studies on the long-term effects on both safety and efficacy of cryopreserved oocytes are lacking due to the relative novelty of these techniques. Safety is unclear (add more details from the oocyte cryopreservation section) no long-term studies.
- **Patient perception;** Elective oocyte cryopreservation is often perceived (and marketed) as a form of insurance, and this could give women a false sense of security and alter behaviour (i.e. encourage women to delay childbearing in the belief they will be able to have children from their stored oocytes).
- **Risks to the future child**; There could be long-term consequences of oocyte cryopreservation on health of the child and possible, as yet unspecified, psychological effects.
- **Funding of these procedures**; It is unlikely that these 'elective' techniques for healthy women will be funded by state health provision/insurance. Funding availability will depend on the healthcare system, but it is unlikely that any system will provide adequate funding for all those who might want to access elective oocyte cryopreservation. Women should

be informed of all costs involved, including for ongoing storage and later use of their oocytes.

- **Ethics;** There are also ethical issues raised by companies offering to pay for women to cryopreserve their oocytes, such as coercion and manipulation, that might make women may feel that they are not able to take time off to have children (Goldman and Grifo, 2016).

Consent and counselling procedures

The ASRM (2018) stresses that women should be told about the novelty of the procedure and uncertainties surrounding it (2018). Psychological counselling (in addition to counselling specific to FP) is usually offered to fertility patients and should be routinely offered to those considering elective oocyte cryopreservation.

There is a clear need to make sure consent processes are robust, so women are aware of:

- Success rates in general and for each stage of the procedure (e.g. success rates for successful oocyte pick-up, storage, thawing and pregnancy rates)
- Novelty of the procedure
- Long-term storage (cost, regulations, usage of stored material, continuation of storage)
- Psychological aspects of the process
- What the procedure involves
- Possible obstetric complications of delayed pregnancy
- Information on the destiny of remaining stored material

Conclusion

Elective oocyte cryopreservation is recognised internationally as an acceptable option to offer women with appropriate cautions and safeguards

Recommendation

Women considering elective oocyte cryopreservation should be fully informed regarding the success rates, risks, benefits, costs and the possible long-term consequences, both in terms of physical and psychological health.	**STRONG** ⊕○○○

Suitability should be determined on a case-by-case basis.	**GPP**

Justification

Although several organizations have published statements on the acceptability of elective oocyte cryopreservation, controversy still exist on whether it should be offered and to whom. The GDG felt there is insufficient data and arguments for strong statements on the latter and decided to recommend determining suitability on a case-by-case basis. Regarding counselling and information provision for women considering elective oocyte cryopreservation, there seems to be a wide acceptance, even though only supported by consensus statements.

Research recommendation

Future research: data should be collected on numbers of women who return to use their frozen oocytes and pregnancy and live birth rates. The psychological benefits of having frozen oocytes should also be explored, as it could be argued that fertility is preserved even if the oocytes are never used. It could also be explored if better education of both men and women about reproductive lifespan would affect the usage or perceptions of elective oocyte cryopreservation

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D5. Embryo cryopreservation

Embryo cryopreservation is an established clinical method in medically assisted reproduction (MAR), and it was the only clinical method for fertility preservation (FP) of adult women for many years (Lee *et al.*, 2006). A paradigm shift occurred in 2013 with the recognition that oocyte cryopreservation through vitrification was an additional clinical option for FP and has become the dominant approach for adult women (Loren *et al.*, 2013, 2013, Yasmin *et al.*, 2018). A key aspect is that as the embryos belong to the couple contributing the oocyte and sperm, a woman who separates from her previous partner may not be able to use the embryos later for attempting pregnancy. Counseling for FP should therefore include a discussion on those specific aspects, hence women with partner may have the option to undergo oocyte cryopreservation or to split their oocytes between cryopreservation of oocytes and cryopreservation of embryos (Rodriguez-Wallberg *et al.*, 2019).

The worldwide increasing use of embryo cryopreservation procedures indicate robust safety and efficacy of this procedure in women undergoing treatment for infertility (De Geyter *et al.*, 2018). Several large studies of the health of the children born after transfer of cryopreserved embryos have been conducted. In general, studies of children conceived through MAR indicate that the children may have elevated risks for adverse birth outcomes, although the absolute risk is small (Goisis *et al.*, 2019). It is also likely that some of the increased risks identified are related to the subfertility of the couple rather than the medical interventions (Goisis *et al.*, 2019). The most recent meta-analysis including data on nearly 300,000 children born through MAR treatments collected from 26 studies indicates reduced risks of prematurity, low birth weight and small for gestational age in children born after transfer of cryopreserved embryos, compared to children born after fresh embryo transfers (Maheshwari *et al.*, 2018). However, increased risks of being large for gestational age, having a birth weight >4000 g and also a higher risk of hypertensive disorders during pregnancy were present in the cryopreservation group (Maheshwari *et al.*, 2018). Two recent studies using Danish and Swedish population-based registries have reported excess risks for children born after transfer of frozen embryos, but not after the transfer of fresh embryos (Hargreave *et al.*, 2019, Rodriguez-Wallberg *et al.*, 2020).

Whereas data on reproductive outcomes of embryo cryopreservation in infertile couples are extensive, data on outcomes after embryo cryopreservation for FP are still scarce. The population of women undergoing FP is also more complex than the infertile population, who are otherwise generally healthy, due to the wide range of indications for FP from oncologic indications when a gonadotoxic treatment is needed, to a broad spectrum of benign diseases or genetic conditions predisposing to premature ovarian insufficiency (POI).

Current international consensus recommends attempting embryo cryopreservation before gonadotoxic treatment starts. A few reports of embryo banking after chemotherapy initiation indicate good embryo morphology and kinetics (Dolmans *et al.*, 2005, Rossi *et al.*, 2011), however data on usage of such embryos in pregnancy attempts are minimal (Nakajima *et al.*, 2015).

PICO Question: Is embryo cryopreservation effective and safe for fertility preservation?

Embryo stage at time of cryopreservation and method of cryopreservation

Data on usage and outcomes of embryos cryopreserved for fertility preservation are scarce and the available evidence comes exclusively from MAR treatments for infertility.

A recent meta-analysis of cryopreservation in MAR evaluated the efficacy of vitrification vs slow-freezing for embryo cryopreservation, pooling data from IVF/ICSI studies (Rienzi *et al.*, 2017). The data suggest that vitrification/warming may be superior to slow-freeze/thawing, regarding clinical

outcomes. The CPR per embryo transfer (N=488), combining data from 3 RCTs, was significantly higher after vitrification than slow-freeze (RR 1.51; 95%CI 1.03-2.23). Data analyzed per cycle indicated a borderline statistical significance (RR 1.89; 95% CI 1.00-3.58). However, in the same meta-analysis, the data compiled from 13 cohort studies and additional sub-analyses of only cleavage embryos or only blastocysts did not confirm these differences (Rienzi *et al.*, 2017). A significantly higher LBR per cycle has been previously reported in one RCT after transfer of vitrified vs slow-freeze cleavage stage embryos (RR 2.28; 95% CI 1.17-4.44), but only of borderline significance when analyzed per transfer (Debrock *et al.*, 2015).

Effect of age or previous gonadotoxic treatment

In women attempting FP by embryo cryopreservation, a negative effect of increasing age and/or previous cancer treatment in the outcome of FP cycles is expected. Studies of embryo cryopreservation for FP have shown that women who are older require higher gonadotropin doses for ovarian stimulation, as do women who undergo cryopreservation cycles after chemotherapy treatment (Dolmans *et al.*, 2005, Rodriguez-Wallberg *et al.*, 2019).

Efficacy of cryopreserved embryos for fertility preservation and usage rates

The usage of embryos cryopreserved for fertility preservation has been investigated in several studies, nearly all have been retrospective, and some have covered extensive periods of time. A common feature in these studies is the small number of women who have returned to attempt pregnancy.

An American cohort study compared embryo usage by 222 women with a cancer diagnosis but no diagnosis of infertility who had cryopreserved embryos between 2004 and 2009 vs 48 women who had cancer and infertility and 68 infertile controls without cancer. The usage rates reported were 10.8%, 31.3% and 85.3%, respectively (Luke *et al.*, 2016). Women with cancer also waited longer to return compared to the control group (Luke *et al.*, 2016). Another retrospective study covering 15 years of FP in the UK reported on 42 women attempting to cryopreserve embryos, of whom 39 women succeeded (Barcroft *et al.*, 2013). Five women returned to undergo FET cycles and 2 live births were obtained (LBR 22% per replacement cycle, but only 4.8% per woman initiating treatment), whereas 3 women conceived naturally (7.1%), 2 couples separated (4.8%) and 14 women discarded their embryos (33%). At the time of the report most women still had embryos stored (Barcroft *et al.*, 2013).

In a French multicenter study 14 centers reported 56 cycles aiming at embryo banking between 1999-2011. Indications included cancer in about 70% of the cases and benign diseases in the remaining (Courbiere *et al.*, 2013). A mean of 4 embryos were frozen per cycle, with 60% of embryos frozen at 2PN zygote stage, 20% at cleavage stage and 2% at blastocyst stage. Ten couples returned to use their embryos and 25 embryos were transferred resulting in CPR of 36% and LBR of 27% per couple (Courbiere *et al.*, 2013). A Belgian study of 52 women who cryopreserved embryos for FP between 1997 and 2014 reported that 23% of women returned to use their embryos. Nine women underwent FET and 6 pregnancies were obtained, with a LBR per patient of 44%, or 11.5% per woman in the cohort (Dolmans *et al.*, 2015).

A UK study reported on 22 women that attempted pregnancy using cryopreserved embryos from a cohort of 531 women undergoing FP over a 15-year period. Although the number of retrieved oocytes was lower in women with gynecologic malignancies compared with those with hematologic malignancies or breast cancer, the fertilization rate and the number of cycles cancelled was similar between the groups. A mean of 7.5 embryos was cryopreserved per cycle, using slow-freeze methods. The PR per transfer cycle was 43.75% and CPR per patient was 54.5% but the miscarriage rate was high resulting in a LBR per patient of 22.7% (Alvarez and Ramanathan, 2018).

[94]

A Swedish prospective study of 562 adult women who had undergone embryo or oocyte cryopreservation for medical indications over a 20-year period, found a return rate of 27% of patients, regardless of benign or malignant indication (Rodriguez-Wallberg *et al.*, 2019). The return rate of women who had completed at least one-year follow-up after FP through embryo cryopreservation was 29%, with CPR and CLBR of 66% and 54%, respectively. However, a significantly lower CLBR after warming cycles was found in women with previous oncologic indication vs women that underwent FP for benign indications (LBR 21% vs. 47%) (Rodriguez-Wallberg *et al.*, 2019). The age of the women who returned was also significantly higher in the group with an oncologic indication vs benign indication at time of attempting pregnancy. These data are consistent with a large retrospective study of usage of vitrified oocytes showing a lower CLBR in 80 women with previous oncologic indication vs 641 women wo underwent elective oocyte vitrification (41.1 vs 68.8%, respectively) (Cobo *et al.*, 2018).

Choice of cryopreservation of oocytes versus embryos.

The comparison of oocyte versus embryo cryopreservation is discussed in section D3. Oocyte cryopreservation.

Safety and risks

Fertility preservation cycles should be considered only in women with no obvious contraindication for ovarian stimulation and/or oocyte pick-up. The risks associated with ovarian stimulation are discussed in the section D2. Ovarian Stimulation .

Recommendation

Embryo cryopreservation is an established option for fertility preservation.	**STRONG** ⊕⊕◯◯
Women should be informed about the risk of losing reproductive autonomy and possible issues with ownership of stored embryos.	**GPP**
Women should be informed of accurate, centre-specific expertise and live birth rates. They should also be informed that success rates after cryopreservation of embryos at the time of a cancer diagnosis may be lower than in women without cancer.	**GPP**

Justification

Embryo cryopreservation is an established technique in infertile couples, and it seems to be effective and safe for women undergoing FP. Regarding efficacy, live births after embryo cryopreservation for FP have been reported, but long-term follow-up data of the children born are not available. The risks associated with embryo cryopreservation are linked to ovarian stimulation and oocyte pick-up, and as such are likely to be similar to the risks of oocyte cryopreservation. The decision on whether to apply embryo or oocyte cryopreservation should be based on considerations of ownership of the resulting embryos and on the success rates of the lab . Furthermore, local legislation will need to be considered, and possible issues with ownership of embryos (as discussed in section D3. Oocyte cryopreservation). The GDG decided to formulate a good practice point recommending women to be informed of the latter risks regarding ownership.

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D6. Ovarian tissue cryopreservation

Ovarian tissue cryopreservation (OTC) can be offered as an alternative to preserve fertility in young patients at risk of premature ovarian insufficiency (POI). Clinical application was supported by large animal experimentations in the 1990's demonstrating the efficacy of the procedure to restore ovarian function and fertility (Anderson and Baird, 2019). OTC is still considered as experimental in many countries, and legislations and regulations vary. However, recently the American Society for Reproductive Medicine (ASRM) suggested to consider it as an established option for selected patients (Practice Committee of the American Society for Reproductive Medicine (2019).

The OTC technique has the advantages of being feasible within a short time frame in both post- and pre-pubertal patients and does not require any preceding drug treatment. The success of the procedure was demonstrated several years after first storage (Donnez *et al.*, 2004), and its use as an alternative to oocyte/embryo cryopreservation (or in combination) has developed rapidly over the last 2 decades.

The procedure of OTC requires high quality control assurance including specific laboratory training distinct from that in 'standard' ART and an appropriate medical environment involving multidisciplinary teams (Andersen *et al.*, 2018). Furthermore, there are specific regulatory aspects relating to tissue rather than gamete storage, and in some countries, ethical committee approval is required, for children, adults or both. Regarding regulatory-legal issues, the procedure has a complex dual nature as "endocrine organ" and "gamete" storage. While organ transplant legislation is usually applied, issues related to ART may also have to be considered, depending on the specific legal situation in the country.

Although research in this field is developing rapidly, the only current option to restore fertility by using cryopreserved ovarian tissue remains ovarian tissue transplantation (OTT). The limitations of OTT are also discussed in this chapter. After OTT, patients can attempt natural conception, or standard ART procedures can be applied. If OTT fails, a second and third tissue replacement can be performed (Gellert *et al.*, 2018).

PICO Question: Should ovarian tissue cryopreservation versus no intervention be used for fertility preservation?

Success rates in patients with cancer and benign conditions

Patient selection criteria and indications vary by centre offering OTC. Formalised recommendations regarding the indications for OTC, i.e. the Edinburgh criteria, included patients younger than 35 years old with >50% of risk of chemotherapy-induced ovarian failure, no previous gonadotoxic treatment, no surgical contraindication and a realistic chance of survival (Anderson and Wallace, 2011, Wallace *et al.*, 2014). Very similar criteria are supported in a recent review (Donnez and Dolmans, 2017). In US, the Oncofertility consortium consensus statement recommends the procedure for patients aged up to 42 years who could or did not want to cryopreserve oocytes or embryos (Backhus *et al.*, 2007). OTC has been performed in patients aged up to 40 or even 49 years by others (Lotz *et al.*, 2016, Jadoul *et al.*, 2017, Karavani *et al.*, 2018). However, pregnancies have been rarely observed when OTC is performed in women older than 35 years and none have been reported after 38 years (Gellert *et al.*, 2018). One paper has compared the reproductive outcomes after OTC with oocyte cryopreservation and confirmed the superiority of oocyte vitrification for patients over 36 years old. In that study, none of the patients over 36 years old at the time of OTC achieved pregnancy while 30% of the patients who achieved pregnancy after using vitrified oocytes were older than 36 years old at the time of FP procedure (Diaz-Garcia *et al.*, 2018). The study reported similar success rates in terms of fertility restoration for OTC and oocyte vitrification in younger patients.

More than 80% of the patients referred for OTC are patients scheduled to receive gonadotoxic therapy, i.e. chemotherapy or radiotherapy for cancers, including haematological (lymphoma or leukaemia) and solid malignancies (breast cancer, sarcoma) (Anderson and Wallace, 2011, Gellert *et al.*, 2018). Other indications include benign conditions that potentially affect the ovarian reserve either due to the disease itself, such as genetic disorders (Turner syndrome, galactosaemia), or due to gonadotoxic treatments, such as alkylating agents for autoimmune disorders (systemic lupus erythematous [SLE]) or as a conditioning regimen before haematopoietic stem cell transplantation (HSCT) (in sickle cell anaemia, thalassaemia)(Condorelli and Demeestere, 2019).

The advantages of OTC include the possibility to restore natural ovarian function (including non-reproductive endocrine effects) after ovarian tissue transplantation (OTT) and to achieve (several) natural pregnancies without further medical intervention. By late 2018, a total of 131 pregnancies have been reported in the literature, resulting in 93 children born (Gellert *et al.*, 2018). More than 85% of the patients showed restored ovarian function within an average period of 4 months after tissue transplantation (range from 1-8 months). The success rate of OTC -defined as at least one child per transplanted patient- was estimated to be around 40% (Pacheco and Oktay, 2017, Gellert *et al.*, 2018). In contrast to established MAR research practice, data are not generally presented per patient starting the intervention (i.e. from the time OTC is first discussed). Overall, the usage rate of cryopreserved ovarian tissue remains low (Diaz-Garcia *et al.*, 2018, Hoekman *et al.*, 2020) but may increase with time.

The ovarian tissue cryopreservation (OTC) procedure

Either an ovarian cortex biopsy (the location of the primordial follicle pool) or one whole ovary can be retrieved at any time during the menstrual cycle and the cortex cryopreserved for future restoration of ovarian function. Where needed, the surgery can be performed in the referring hospital and the ovarian tissue transported (1 to 20h) under strict conditions to a qualified fertility clinic laboratory/tissue bank for processing and cryopreservation (Andersen *et al.*, 2018). A review of 455 OTC procedures, for which details on the surgical procedure were available, showed that laparoscopy is the most commonly used approach to collect the tissue, although mini-laparotomy was also described in children (Beckmann *et al.*, 2016, Corkum *et al.*, 2017). Several centres perform ovarian biopsy (1/3 to 2/3 of one ovary), while others routinely perform unilateral oophorectomy (Beckmann *et al.*, 2016). Oophorectomy by single-incision laparoscopic surgery was shown not to be inferior to standard 2- or 3-port laparoscopy in terms of complication rate, duration of the procedure, hospital stay and delay to start chemotherapy (Karavani *et al.*, 2018). Although reduced-port laparoscopy is feasible and less invasive, it requires a learning curve and should not be offered in case of pelvic diseases such as endometrioma or fibroma (Kikuchi *et al.*, 2013, Karavani *et al.*, 2018). As such, this technique can be offered by trained surgeons in the absence of pelvic disease.

For ovarian biopsy, large fragments of cortex at a distance from the hilum and from any large visible follicles or corpus luteum should be harvested and careful haemostasis should be achieved after tissue removal (Corkum *et al.*, 2017). An advantage is to maintain two ovarian sites for future transplantation and to limit the invasiveness of the procedure, given the uncertainty over loss of ovarian function from the proposed chemotherapy in many cases. While there is no evidence that having one ovary affects the fertility potential of patients who recover normal ovarian function after OTC (Schmidt *et al.*, 2013), population-based data have shown that the time to menopause is shortened in women who underwent unilateral oophorectomy compared to controls (adjusted relative risk [RR] 1.27; 95% CI 1.14-1.41) (Bjelland *et al.*, 2014). There are no comparable data relating to women who have undergone chemotherapy in addition to unilateral oophorectomy. If the remaining ovary remains functional, another risk is the possibility of inducing POI if any gynaecological disease such as ovarian torsion, endometriosis, or borderline tumour is later diagnosed and requires radical surgery.

No difference in the complication rate has been reported between the two approaches (Corkum *et al.*, 2017). Overall, complications related to OTC procedures are rarely reported irrespective of the technique. In a large series of 545 cases of OTC, five minor complications and one major event were

reported (Jadoul *et al.*, 2017). In another cohort of 225 patients, one severe complication was reported during anaesthesia, leading to the patient's death (Imbert *et al.*, 2014).

At the laboratory, the tissue is dissected under sterile conditions to obtain small fragments of cortex of around 1 mm thickness (< 2mm is required for effective cryopreservation). The large majority of primordial follicles are detected at less than 1mm below the surface of the cortex and the localization does not change with age in adults, although some primordial/primary follicles were found at 1.5 mm depth in POI patients (Haino *et al.*, 2018). Each fragment can be cryopreserved individually for long-term storage using slow-freezing or vitrification techniques (these options are discussed in detail below). Analysis of tissue stored for 18 years showed that long storage did not affect follicular morphology and survival (Fabbri *et al.*, 2016a). Cryopreserved ovarian tissue using the slow-freezing procedure and stored for more than 14 years has been transplanted with success (Gellert *et al.*, 2018).

The ovarian tissue transplantation (OTT) procedure

Ovarian tissue transplantation (OTT) can be performed at heterotopic and/or orthotopic sites. Orthotopic transplantation into the remaining ovary, broad ligament, or ovarian peritoneal pocket is the most common procedure (Gellert *et al.*, 2018). There is no evidence for the superiority of one orthotopic site over the others in terms of endocrine and reproductive outcomes and they are often combined. The evidence is from case series and reports thus comparisons (in the absence of patient-specific issues) have little validity. After auto-transplantation of ovarian tissue at an orthotopic site, more than 60% of pregnancies occurred after natural conception in patients treated for cancer or benign conditions (Gellert *et al.*, 2018). Pregnancies obtained after transplantation at the peritoneal site usually required IVF treatment (Gellert *et al.*, 2018), although it is unclear whether patient/medical preference contributed to that. For patients with specific ovarian risks (such as BRCA mutation carriers), the decision regarding the site of transplantation should also take into consideration the need to remove the grafted ovary after pregnancy (Lambertini *et al.*, 2018). Transplantation at the heterotopic site, such as subcutaneously in the forearm or to the abdominal wall, is less invasive and efficient to restore endocrine function (Bystrova *et al.*, 2019). However, only one live birth has been reported so far after transplantation to the anterior abdominal wall (Stern *et al.*, 2013, Stern *et al.*, 2014).

Patients who succeeded in conceiving after OTT were younger at OTC than those who did not (26.4 ± 6.3 versus 29.6 ± 5.4 years) (Gellert *et al.*, 2018). Another review (based on similar studies and reports) showed no significant difference in age between patients with restored ovarian function or not (28.5 ± 6.0 versus 31.0 ± 10.0 years) (Pacheco and Oktay, 2017).Other factors that could affect the success rate after OTT are the amount of transplanted tissue and the follicular density (Poirot *et al.*, 2019). One group has described criteria based on the ovarian reserve under which OTC should not be performed, considering the unfavourable risk/benefit balance. These criteria are based on the 5th centile of AMH and AFC in cancer patients younger than 35 years (0.4ng/ml and 5 visible follicles, respectively) (Paradisi *et al.*, 2016).

The mean graft longevity from the time of OTT was 24.9 months but with large variation (range 4-144 months). Up to 3 pregnancies and live births in an individual patient have been reported in a period of more than 7 years after OTT. There is no generally accepted upper age limit for the OTT procedure, and it has been performed in women up to 47 years old (Gellert *et al.*, 2018), although issues around maternal risks in pregnancy are important and should be considered (see E2. Obstetric outcomes).

Consensus is lacking regarding the amount of the tissue that should be replaced to optimize the chance of pregnancy after OTT. A meta-analysis of 309 OTT procedures in 255 women showed that 1/3 of the ovary was usually used for grafting but also reported that 45 patients required two OTT procedures to achieve pregnancy (Pacheco and Oktay, 2017). A third OTT has been offered in less than 1% of patients (Gellert *et al.*, 2018). The data suggest that sufficient amount of tissue can be obtained from 1 or 2 large biopsies but no evidence of the superiority of any of the approaches in terms of outcomes has been demonstrated, and it is likely that the inherent variation in the follicle

density between individual women is a major determinant. The decision on the amount of tissue to replace should take also into consideration surgical limitations and the amount of tissue available for transplantation (surgeon's experience, ovarian transplantation sites). More tissue may be required to restore ovarian function in patients with a low ovarian reserve.

Recommendation

OTC is an effective method for ovarian function and fertility preservation. It is recommended to offer OTC in patients undergoing moderate/high risk gonadotoxic treatment where oocyte/embryo cryopreservation is not feasible, or at patient preference.	**STRONG** ⊕○○○

OTC should probably not be offered to patients with low ovarian reserve (AMH<0.4ng/ml and AFC<5) or aged above 36 years considering the unfavourable risk/benefit	**WEAK** ⊕○○○

Justification

Based on the reported data of OTC and OTT, summarized in reviews and meta-analysis of observational data, the procedure is effective in restoring fertility with reasonable chances of achieving a live birth (Pacheco and Oktay, 2017, Gellert et al., 2018). Data also suggest that OTC, and more specifically the retrieval of ovarian tissue, is to be considered safe, although general risks of surgery need to be considered. For patients undergoing moderate/high risk gonadotoxic treatment where oocyte/embryo cryopreservation is not feasible, the benefits of OTC seem to outweigh the risks. Patient preference could be another factor in decision-making.

OTC is feasible and acceptable, although the surgeon should acquire the necessary skills, and the lab require specific competence which is not readily available in ART labs, including equipment and specific SOPs. Legal restrictions and/or the need for ethical approval should be considered as well.

Data on the efficacy of the procedure to restore fertility have shown that there is a significant impact of the age of the patient and the ovarian reserve. Regarding age, a threshold of 36 years seems appropriate (Gellert et al., 2018). For women over 36 years, oocyte cryopreservation was found to be superior to OTC (Diaz-Garcia et al., 2018). With regards to ovarian reserve, the study from Paradisi and colleagues, provides thresholds for AMH and AFC (Paradisi et al., 2016). For these patients (over 36 years and/or with low ovarian reserve), the risks of the procedure may outweigh the limited benefits, and the GDG therefore suggests using other FP interventions.

Recommendation

The GDG recommends that OTC is considered to be an innovative method for ovarian function and fertility preservation in post-pubertal women.	**GPP**

Justification

OTC is considered effective in restoring fertility in post-pubertal patients (Pacheco and Oktay, 2017, Gellert et al., 2018) , although the data on technical variability, efficacy and safety are still limited. With data of proof-of-principle, the technique should be categorized as "innovative" but not yet as "established" as this would require long-term safety data, and evidence of procedural reliability and high effectiveness, (Provoost et al., 2014). Recommendations for innovative treatments should be followed (Provoost et al., 2014), including monitoring of data, informing patients, and performing the procedure only in centres with appropriate expertise. For the latter, recommendations on key technical aspects are outlined below. Before OTC can be considered an established (or standard) procedure, more data should be available, mainly on the effectiveness in restoring fertility and long-term safety for patients and their children.

Key technical aspects of OTC

There It is necessary to have appropriate equipment, quality control and training for the health care team before performing OTC.

Local legal aspects should also be taken into account, including the need for ethical approval.

Laparoscopy caries a low risk (in healthy women) and is considered as the standard surgical procedure to collect the ovarian tissue. However, patients referred for OTC may have an increased risk of surgical complications.

It is possible to perform the laparoscopy in the referring centre and transport the tissue under strict condition for up to 20 hours before processing.

Both unilateral oophorectomy and biopsy are acceptable for collecting ovarian tissue. The choice will depend on the patient characteristics, their scheduled treatments, and available expertise in the centre. In the majority of patients, removal of two-third of the ovarian cortex surface from one ovary is sufficient to achieve pregnancy.

Impact of ongoing treatments and previous history of chemotherapy on OTC procedure

One of the major advantages of OTC compared to oocyte/embryo cryopreservation is the possibility to perform the procedure after starting chemotherapy treatment. Patients may benefit from a first line regimen before OTC or can be referred for OTC before consolidation therapy such as conditioning regimen for HSCT after a limited response to low gonadotoxic regimen such as standard ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) for Hodgkin lymphoma.

The interval between OTC and the last chemotherapy treatment has to be taken into consideration for the evaluation of ovarian reserve testing. The AMH level dramatically falls within 2 weeks after gonadotoxic treatment, even after low gonadotoxic treatment initiation (ABVD), and recovery takes usually at least 6 months (Peigne and Decanter, 2014). The follicular density in ovarian samples collected before and after first line chemotherapy in young cancer patients was similar and irrespective of the interval between chemotherapy and OTC (from <1month to years) (El Issaoui *et al.*, 2016). A higher follicle density after ABVD has even been reported in lymphoma patients who performed OTC within an interval of 1 to 36 months after chemotherapy completion (McLaughlin *et al.*, 2017). However, a lower proportion of intact follicles was observed before and after in vitro culture when the tissue was previously exposed to chemotherapy (46% and 6% before and after culture in exposed tissue, versus 82% and 28% in non-exposed tissue)(Asadi Azarbaijani *et al.*, 2015). After a first-line chemotherapy with higher doses of alkylating agents (median cumulative dose of 6100mg/m² Cyclophosphamide Equivalent Doses (CED 0-20,840) and 205mg/m² Doxorubicin Isotoxic Equivalent doses (DIE 90-450)) in patients aged less than 25 years, a significant effect on the follicular density and atresia has been recently reported, suggesting the need for more data on the efficiency of the procedure in this context (Pampanini *et al.*, 2019).

In a recent study in women receiving a first line low gonadotoxic regimen (n=22), there was no difference in ovarian function recovery rate nor pregnancy rate after OTT compared to patients who did not received any treatment before OTC (n=9) (Poirot *et al.*, 2019). Furthermore, there was no difference when OTC was performed >3 months after or during chemotherapy. Nevertheless, additional data are required regarding the safety of OTT using ovarian tissue previously exposed to chemotherapy and the time required for DNA repair or other processes in exposed oocytes.

Recommendation

Young patients who have already received low gonadotoxic treatment or a previous course of chemotherapy, can be offered OTC as FP option.	WEAK ⊕OOO

Justification

Although based on a small cohort, results show no effect of previous low gonadotoxic chemotherapy on ovarian function recovery rate nor pregnancy rate after OTT (Poirot et al., 2019). Furthermore, for patients who previously received low gonadotoxic treatment, OTC may be their only option for FP.

OTC in a combined procedure

OTC has been combined with oocyte/embryo cryopreservation after ovarian stimulation. In 2 series reporting a total of 28 patients, ovarian stimulation was performed after OTC to increase the chance of future pregnancy by additional oocyte cryopreservation (Huober-Zeeb *et al.*, 2011, Dolmans *et al.*, 2014). Ovarian stimulation was started between 1-2 days before and 1-3 days after laparoscopy. The authors did not observe any (significant) difference in the duration of stimulation, the number of oocytes collected, or the number of good quality embryos obtained compared to infertile patients or FP patients who did not have OTC. These studies did not report adverse events.

In another study, OTC was performed on the same day as oocyte pick-up in 14 patients (Dittrich *et al.*, 2013). The authors reported uneventful transvaginal oocyte pick-up in all cases without perioperative bleeding complications. The ovarian grafts had a normal histological appearance and a normal follicular count. Data on outcomes after transplantation are not available.

OTC can be also be associated with ovarian transposition in patients who will be treated with pelvic irradiation (Aubard *et al.*, 2001). A recent case report showed the combination of OTC with ovarian transposition and GnRH agonist protection is feasible and effective (with regards to endocrine function). Pregnancy data were not reported. Further details on ovarian transposition are covered in section D9. Ovarian transposition.

Recommendations

Ovarian stimulation can be performed immediately after OTC.	WEAK ⊕OOO
OTC at the time of oocyte pick-up after ovarian stimulation should not be performed unless in a research context.	RESEARCH ONLY
Ovarian transposition can be performed at the same time as OTC in patients who will receive pelvic irradiation	GPP

Justification

To increase the chances of future pregnancy, OTC can be combined with other FP strategies. The combination of OTC with oocyte cryopreservation seems feasible and effective, but this conclusion is based on very limited data on efficacy, without data of pregnancies or births.

Performing oocyte pick-up on the same day as laparoscopy for OTC (with reducing the need for anaesthesia) also seems to be feasible, but there is even less evidence. As such, this can only be performed in a research context until data (on safety) are available.

Ovarian transposition at the same time of OTC is feasible and theoretically it does not have increased risks in comparison to OTC or ovarian transposition as single therapy.

OTC for other indications

Transgender men

Although oocyte or embryo cryopreservation is the recommended FP method in transgender men, OTC can be performed especially as the ovaries removed in gender reassignment surgery can be cryopreserved without the need for further interventions. However, use of the cryopreserved tissue would require replacement in the transman, thus a full discussion should be undertaken. There are no studies evaluating the effectiveness and the safety for later use of cryopreserved ovarian tissue in this population (Baram *et al.*, 2019).

Recommendation

OTC is not recommended as primary FP procedure in transgender men but can be proposed as an experimental option when ovaries are removed during gender reassignment surgery	**GPP**

Justification

There are no studies evaluating the effectiveness and the safety of OTC/OTT in transgender men. An important consideration in this patient group is the acceptability of ovarian tissue auto-transplantation. Based on these considerations, OTC/OTT should not be recommended as an FP method for transgender men, when other options are available. In the future, however, stored ovarian tissue may be used in combination with in vitro growth and therefore cryopreservation of tissue from ovaries removed in gender reassignment surgery can be considered.

Genetic disorders

OTC has been offered also in young patients (often children) with genetic disorders when there is an associated risk of POI such as in galactosemia, Turner syndrome and Blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES) syndrome. At present, OTT has not been performed to attempt to restore fertility in patients with POI-associated genetic disorders. The procedure should be offered within a clinical research protocol and requires a multidisciplinary approach including genetic counselling (Anderson and Baird, 2019, Condorelli and Demeestere, 2019).

Allograft between identical sisters has also been reported in the unusual situation where one has developed POI. These indications will not be discussed in the present guideline but are at present not recommended considering the unfavourable risk/benefit balance compared to other well-established alternatives.

Recommendation

OTT can be considered in patients with POI-associated genetic and chromosomal disorders but requires genetic counselling and should be performed within a research protocol.	**RESEARCH ONLY**

Justification

To our knowledge, OTT has not been performed to attempt to restore fertility in patients with POI-associated genetic disorders. In absence of data on safety or efficacy, such OTC/OTT for these patients should be performed in a research context. For these patients, the risk of transmission of the genetic disease to the offspring is a major concern and genetic counselling is recommended.

Other considerations

OTC has also been suggested an option for elective fertility preservation or for delaying the menopause (Yding Andersen *et al.*, 2019); while full discussion of this application is outwith the remit of this guideline, the ethical issues as well as the balance of risk/benefit remain questionable and this approach is currently not recommended. The transplantation of the cryopreserved ovarian tissue for other indications such as pubertal induction and endocrine function restoration has been reported but remains controversial.

Vitrification versus slow-freezing

The protocol most widely used for ovarian cryopreservation is slow-freezing and rapid thawing of the ovarian tissue. Vitrification has been widely implemented in fertility laboratories as a standard method for cryopreservation of embryos and oocytes and has been suggested as an alternative technique for OTC. Vitrification has several potential advantages, as it avoids cell damage induced by ice formation, it is less time-consuming, and it does not require an expensive controlled rate freezer (Shi *et al.*, 2017). Here we consider the evidence from human and primate studies comparing the two techniques.

PICO Question: Should vitrification versus slow-freezing be used for ovarian tissue cryopreservation for fertility preservation?

The most important parameters for clinical application remain the developmental capacity of the oocytes within follicles grown after auto-transplantation of frozen-thawed tissue, and the clinical outcomes. There are no studies evaluating these criteria where the two techniques are compared.

Published cases of the transplantation of frozen-thawed ovarian tissue were recently summarized. Eighty seven live births were reported, but data on health of the baby were only available for 40 births (Gellert *et al.*, 2018). All these children were born healthy, except one who was affected by foetal arthrogryposis. In the review, the authors did not detail the cryopreservation technique. In another review, Shi and colleagues identified only two live births using vitrified ovarian tissue (Shi *et al.*, 2017). In the meta-analysis of Pacheco and Oktay (also 2017), 19 reports were included with a total of 309 OTTs in 255 patients. In all live births reported, the tissue was stored using slow-freezing (Pacheco and Oktay, 2017).

A recent meta-analysis by Shi compared the efficiency of ovarian tissue vitrification with slow-freezing (Shi *et al.*, 2017). Fourteen non-randomized studies were included, and four parameters were considered for the analysis: the proportion of intact follicles (10 studies), DNA fragmentation in primordial follicles (6 studies), the proportion of normal stromal cells (6 studies) and the primordial follicle density (3 studies). There was high heterogenicity regarding the protocol for vitrification between studies. The proportion of intact primordial follicles and the follicular density were similar between the two techniques, while DNA damage was less frequently observed in the vitrification group (RR 0.71; 95% CI 0.62–0.80). Stromal cells also showed less damage in the vitrification group (RR 1.69; 95% CI 1.47–1.94). In contrast, Dalman et al. showed that expression of apoptotic markers, excluding CASP3, were significantly higher after vitrification than slow-freezing (245 follicles after vitrification, 175 follicles after slow-freezing, and 272 follicles in control were analysed) (Dalman *et al.*, 2017). Fabbri et al. evaluated mitochondrial activity (not reported in the review) and reported that it was better preserved in the slow-freezing group (Fabbri *et al.*, 2016b).

No difference in follicular morphology or viability were observed between vitrified and slow-frozen-thawed ovarian tissue after 8 days of in vitro follicular culture (Wang *et al.*, 2016). After xenotransplantation of human ovarian tissue into mice, no difference in the vascularization or fibrosis were reported between the 2 procedures in most of the studies although there were some discrepancies according to protocols (Rahimi *et al.*, 2010, Amorim *et al.*, 2012, Herraiz *et al.*, 2014, Abir *et al.*, 2017, Lee *et al.*, 2019).

Experiments in non-human primates showed that secondary follicles and stroma cells were better preserved with vitrification compared to slow-freezing (Ting *et al.*, 2011). However, follicular growth occurred irrespective of the cryopreservation techniques after long-term grafting (Dolmans *et al.*, 2015).

Recommendations

The slow-freezing protocol for OTC is well-established and considered as standard.	**STRONG ⊕○○○**
Vitrification of ovarian tissue should only be offered within a research program.	**RESEARCH ONLY**

Justification

The slow-freezing protocol for OTC is considered to be well-established, as it was used in the large majority of data on OTC. Slow-freezing is considered feasible.

Vitrification of ovarian tissue is a promising technique, supported by technical aspects. However, the number of live births after replacement of vitrified tissue is very limited, and there is a lack of consensus regarding the optimal protocol. Therefore, the GDG recommends for vitrification of ovarian tissue to be performed only in a research-context awaiting further data.

Replacing ovarian tissue: safety concerns

PICO Question: Which safety issues should be considered when replacing ovarian tissue?

At present, reports show that more than 300 patients have had ovarian tissue replaced, resulting in 131 pregnancies and 93 children born (Gellert *et al.*, 2018). Before replacing tissue, the balance between the risk and the benefit should be carefully evaluated by a multidisciplinary team. The safety issues include:

- the surgical complications
- the risk of reintroducing malignancy
- the oncological outcomes in hormonal-sensitive diseases
- the risk for offspring.
- the long-term risk of OTT

Surgical complications

OTT at the orthotopic site, either on the remaining ovaries or in the nearby peritoneal site, is usually performed by laparoscopy, and more rarely by mini-laparotomy, under general anaesthesia (Schmidt *et al.*, 2011, Beckmann *et al.*, 2017). The patient can be discharged on the same day or the day after surgery. Drainage tubes were required in less than 50% of the cases (Schmidt *et al.*, 2011, Beckmann *et al.*, 2017). No complication after OTT has been reported so far, except one switch to laparotomy for extensive adhesions (Beckmann *et al.*, 2018). The surgical procedure was considered to be at very low risk of complications (around 1%), similar to ovarian tissue removal procedure (Beckmann *et al.*, 2017, Beckmann *et al.*, 2018). The transplantation procedure is usually performed in one step laparoscopy using standard or robot assisted techniques. A two-step laparoscopy (one week interval) to prepare the transplantation site and induce neovascularization has been proposed (Donnez *et al.*, 2004, Demeestere *et al.*, 2009) but is not widely used, and there is no evidence for the superiority of the two-step procedure in terms of ovarian function recovery and pregnancy rate. Robot-assisted laparoscopy has been reported, but only in case reports (Demeestere *et al.*, 2015, Oktay *et al.*, 2016, Oktay *et al.*, 2019). Surgery can be combined with other

procedures including hysteroscopy, assessment of the patency of the fallopian tubes, or other gynaecological surgery as required according to the clinical context (Beckmann *et al.*, 2018).

OTT at heterotopic sites such as subcutaneous or other extrapelvic sites is less invasive but requires an ART procedure to attempt pregnancy and success rate is limited (*see OCT outcomes above*).

As for the OTC procedure, a quality control system is mandatory during the thawing procedure and the transfer of the ovarian tissue to the operative room, which should be close by (Andersen *et al.*, 2018). Although no infection has been described, bacteriologic assessment of the media used for cryopreservation, thawing and transport should be part of the quality control process, and prophylactic antibiotic administration during the surgery should be considered.

Recommendations

A standard laparoscopy procedure for OTT is considered safe without causing additional surgical risk	**STRONG** ⊕⊕○○

OTT at the orthotopic site is recommended to restore fertility	**STRONG** ⊕⊕○○

Justification

We did not find any reports of severe surgical complications linked to ovarian tissue transplantation, except for one intraoperative switch to laparotomy (Beckmann et al., 2018). Recent reviews also confirmed that the procedure is considered safe. Laparoscopy and replacement at the orthotopic site are often used, and as such most data on efficacy and safety of OTC and OTT are based on these procedures. Transplantation at the orthotopic site furthermore has the advantage of possible natural conception, whereas heterotopic transplantation requires ART. Therefore, laparoscopy and replacement at the orthotopic site seem to be the preferred option when transplanting ovarian tissue for restoration of fertility (Beckmann et al., 2017, Gellert et al., 2018). OTT surgery and thawing of the ovarian tissue should be performed at the same centre.

Risk of reintroducing malignancy

Ovarian metastases have been reported in more than 20% of female autopsies from non-gynaecological malignancies, both haematological and solid tumours. In cancer patients, the risk of the presence of residual cancer cells in the cortex should always be carefully evaluated using the most sensitive techniques, according to the disease. These may include immunohistology, molecular markers and/or a xenograft model when available. Before OTT, the patient should be in good health and free of the disease for a sufficient period which will vary according to the type of cancer and the stage (Andersen *et al.*, 2018). Information regarding the oncological follow-up should be reported at least during 2 years after OTT in order to evaluate the involvement of grafted ovarian tissue in a possible relapse (Andersen *et al.*, 2018). A multidisciplinary approach is mandatory to evaluate the safety of the procedure, as well as providing clear information to the patient.

Ovarian and adnexal tumours

OTT is probably not recommended in patients treated for borderline ovarian tumour (BOT) or ovarian cancer. BOT is bilateral at diagnosis in 15-40% of the cases and if the disease is unilateral at diagnosis, the risk of recurrence in the contralateral ovary remains high. Positive residual tumour in the ovarian cortex has been observed in around 10% of patients with BOT (Masciangelo *et al.*, 2018). In ovarian cancer, the risk of invasive cell contamination in the contralateral ovary is also present. A study analysing fragments of ovarian tissue from 23 patients with ovarian tumours (including adenocarcinoma (n=9), cystic teratoma (n=3), granulosa cells tumour (n=1), dysgerminoma (n=6), endodermal sinus tumours (n=2) and BOT (n=2)) did not reveal the presence of malignant cell

contamination by immunohistological analysis or disease development after xenografting in a mouse model (Lotz *et al.*, 2011). However, data regarding the risk of ovarian tissue involvement in these patients are limited and the detection technique may be not sensitive enough to detect micro-metastasis. Transplantation at the peritoneal site of ovarian tissue collected from the contralateral ovary, free of any cancer cells after analysis, has been described. However, the authors concluded that the peritoneal site is not recommended as it is difficult to completely remove the graft after achieving pregnancy (Kristensen *et al.*, 2017).

Haematological malignancies

Malignant cells were detected by molecular techniques in around half of ovarian tissue samples from patients diagnosed with leukaemia (Dolmans *et al.*, 2013). Postponing OTC to the time of morphological bone marrow remission (after first chemotherapy induced regimen) resulted in less or no leukaemic contamination in the ovarian material (Jahnukainen *et al.*, 2013). Moreover, the viability of the residual leukaemia cells after xenograft in these cases was questionable (Greve *et al.*, 2012). A first case report of successful OTT to restore fertility in a leukaemia survivor using ovarian tissue collected after complete remission and before bone marrow transplantation has been described (Shapira *et al.*, 2018). Thus, OTT can be offered in leukaemia patients if OTC has been performed at the time of bone marrow remission, after careful evaluation of tissue fragments and/or residual medulla using appropriate molecular techniques and xenografting models. There is however no consensus as to what constitutes a comprehensive analysis for safety. Research projects are ongoing to eliminate malignant cells from the ovarian tissue or to offer alternatives (e.g. *in vitro* growth of small follicles, artificial ovary) in order to increase safety (Anderson *et al.*, 2017). However, these approaches are still experimental and not available yet for clinical application (see PART F: Ongoing developments in FP).

Patients with lymphoma make up around a third of OTT procedures (Gellert *et al.*, 2018). OTT is considered to be safe in Hodgkin Lymphoma patients, although ovarian micro-metastasis can occur especially in high stage pelvic disease (Bittinger *et al.*, 2011) (Bastings *et al.*, 2013, Gellert *et al.*, 2018). Ovarian tissue involvement can occur in diffuse large B-cell, Burkitt lymphoma and other lymphoma subtypes (Bastings *et al.*, 2013), indicating that an accurate analysis of the ovarian tissue and an individual multidisciplinary evaluation of the risk for each fertility restoration is required, although there have been no reported recurrences due to OTT.

Other solid tumours

- OTT is probably safe in bone and soft tissue tumours (Dolmans *et al.*, 2016). However, ovarian involvement has been described in patients with Ewing's Sarcoma (Abir *et al.*, 2010, Anderson *et al.*, 2017), although a study found no tumour cell contamination in ovarian tissue from sarcoma patients (Greve *et al.*, 2013). Still, caution should be taken when there is pelvic involvement.
- Medulloblastoma and neuroblastoma are considered at high risk of ovarian involvement although analysis of ovarian tissue at OTC has not shown ovarian involvement (Bastings *et al.*, 2013, Dolmans *et al.*, 2013). No data on OTT in patients treated for cancers of the central nerve system are available.
- Data on OTT risk in breast cancer patients are reassuring (Fabbri *et al.*, 2012). The procedure is considered safe if no pelvic involvement or distant metastasis are observed at the time of OTC although molecular markers are not available or suboptimal. (Bastings *et al.*, 2013) (Luyckx *et al.*, 2013, Bockstaele *et al.*, 2015, Rodriguez-Iglesias *et al.*, 2015).
- For other solid tumours as cervical, gastro-intestinal, colorectal or respiratory cancer, ovarian involvement is rarely described but data are scarce (Bastings *et al.*, 2013). In general, individual assessment should be performed before OTT based on the markers available and the characteristics of the disease.

Recommendations (see also Table 9)

The decision to perform OTT in oncological patients requires a multidisciplinary approach	**GPP**
It is recommended to evaluate the presence of residual neoplastic cells in the ovarian cortex (and in the residual medulla when available) using appropriate techniques in all cancer survivors before OTT.	**STRONG** ⊕○○○
OTT is not recommended in cases where the ovary is involved in the malignancy.	**STRONG** ⊕○○○

Justification

Disease transmission is a major concern in OTT, and although the risks are very much dependant on the type and stage of the cancer, a multidisciplinary discussion of benefits of OTT with regards to fertility, and risks of cancer recurrence is highly recommended for all oncological patients. This is consistent with published recommendations from expert teams (Andersen et al., 2018).

Based on the theoretical risk of disease transmission and the availability of techniques to detect malignant cells in the tissue before transplantation, it seems reasonable to recommend screening of the tissue before OTT (Bastings et al., 2013). Limitations of the current available techniques (for instance with regard to detection of micro-metastasis) should be taken into consideration.

For patients where the ovary was involved in the malignancy, the risk of reintroducing cancer seems to outweigh the benefits of the OTT procedure, and OTT is not recommended. Alternative options as collection of immature oocytes from the tissue may be a safer option (Lotz et al., 2011).

Oncological outcomes in hormone-sensitive diseases

Concern may be raised regarding the risk of recurrence of the disease after OTT procedure and pregnancy in patients with hormone-sensitive tumours. It has been well established that a subsequent pregnancy in women treated for hormone receptor positive breast cancer does not increase the risk of recurrence compared to matched patients who did not have a pregnancy after treatment (HR 0.94; 95% CI 0.70-1.26) (Azim *et al.*, 2013, Lambertini *et al.*, 2018). This is discussed further in section E2. Obstetric outcomes. Similarly, pregnancy did not affect the oncological prognosis of patients treated for melanoma (pooled HR for mortality 0.81; 95% CI 0.60-1.09) (Byrom *et al.*, 2015). Pregnancy may be even a positive prognostic factor in patients with endometrial cancer who benefit from fertility-sparing treatment (Chae *et al.*, 2019).

Recommendation

Hormone-sensitive tumours such as endometrial and breast cancer are not a contraindication for ovarian tissue transplantation (OTT) and pregnancy after complete remission of the disease	**STRONG** ⊕⊕○○

Justification

For patients with hormone-sensitive tumours, concerns have been raised regarding the risk of recurrence due to pregnancy. However, evidence suggests that pregnancy does not have a negative impact on survival in patients with a previous history of a hormone-sensitive tumours (Lambertini et al., 2018), and as such neither OTT nor pregnancy should be considered contraindicated.

503 Table 9 Summary of GDG recommendations for specific patient groups

Disease	Considerations for OTC/OTT	Recommendation for OTT
Ovarian or adnexal tumour	OTC should only be carried out after careful consideration, when other options are not feasible, bearing in mind that replacement may not be available to the patient in the foreseeable future due to the high risk of recurrence and the risk of cryopreserved ovarian tissue involvement.	OTT is probably not recommended considering the high risk of ovarian tissue involvement. The safety of OTT with removal after pregnancy needs to be further investigated
Leukaemia	Ovarian tissue should ideally be collected at the time of complete bone marrow remission (after first chemotherapy regimen) and it should be tested using molecular detection techniques before OTT. If molecular markers are not available, xenograft experiments should be performed.	OTT should be considered with extreme caution considering the high risk of ovarian involvement by leukemia cells
Tumours of the central nerve system (CNS)	Data are limited regarding the risk of reintroducing the disease in patients treated for CNS tumours. Medulloblastoma and neuroblastoma are considered at higher risk.	OTT should be considered with extreme caution. Additional data are needed regarding the safety of OTT.
Non-Hodgkin Lymphoma	OTC/OTT can be performed in patients with non-Hodgkin lymphoma with no evidence of distant metastasis or pelvic involvement at diagnosis.	OTT appears to be safe if pelvic involvement is excluded at diagnosis. OTT can be considered after appropriate ovarian tissue testing using histology and molecular approaches when available
Hodgkin Lymphoma	OTC/OTT appears to be safe in patients with Hodgkin lymphoma when pelvic involvement was excluded at diagnosis.	OTT appears to be safe if ovarian involvement is excluded at diagnosis. OTT can be considered after appropriate ovarian tissue testing using histology
Cervical tumours	Ovarian involvement is rare at diagnosis, and more frequent in adenocarcinoma than in squamous cell carcinoma.	OTT appears to be safe in patients treated with fertility-spearing strategy although more data are requested regarding the risk of ovarian tissue involvement in patients after OTC.
Other solid tumours	OTC/OTT appears to be safe in patients with solid tumour such as sarcoma, breast cancer, gastro-intestinal and colorectal malignancies when distant metastasis and pelvic involvement was excluded at diagnosis. ,	OTT appears to be safe in non-metastatic disease at OCT. OTT can be considered after appropriate ovarian tissue testing, using histology and molecular markers when available

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Risks for the offspring

No evidence of additional risk of congenital abnormalities or genetic disorders after OTT has been reported (Imbert *et al.*, 2014, Gellert *et al.*, 2018). The rate of congenital abnormalities in the children was estimated to be 1.2%, which is comparable to the rate of major malformation occurring in general population (Pacheco and Oktay, 2017). In a recent study on the fertility outcomes after OTT in 22 patients who received first line chemotherapy before ovarian tissue cryopreservation, the authors reported 13 pregnancies in 7 patients, resulting in 8 healthy children (Poirot *et al.*, 2019).

Recommendation

There appears to be no increased risk of congenital abnormalities for children born after OTT	**WEAK ⊕○○○**

Justification

Available data show no increased risk of congenital abnormalities in children born after OTC and OTT (Pacheco and Oktay, 2017, Gellert et al., 2018). However, the number of live births from these procedures remains low and may be insufficient to make reliable conclusions. This is particularly the case when OTC is undertaken after chemotherapy exposure. Therefore, large cohort studies with collection of long-term follow-up data of the babies, including data on congenital and other possible abnormalities in the offspring, are still required.

Long-term risk of OTT

The first patient who underwent transplantation of cryopreserved ovarian tissue dates back almost 20 years ago. Although numbers remain small, no long-term risks of the procedure have been reported so far. Very limited data are available in animals regarding the risk of malignant transformation of transplanted ovarian tissue, especially at the heterotopic sites. Animal studies have shown that ovarian tissue transplantation into a hormonal-sensitive organ such as liver can induce hepatocellular neoplasms (Klotz *et al.*, 2000). In the rat model, granulosa/theca cell tumours were observed during long-term follow-up after transplantation of cryopreserved or fresh ovarian tissue into the spleen (Mueller *et al.*, 2005). The authors suggested that the high level of gonadotrophins stimulated the development of sex-cord tumours in this model. Despite the lack of clinical relevance of these transplantation sites, it raises the question of the long-term outcome of the heterotopic-transplanted tissue in human.

Long-term risks may also be present for patients with breast cancer patients and a germline mutation in BRCA1 or BRCA2 genes. Besides breast cancer occurring often at a reproductive age, BRCA mutation carriers have a high risk of ovarian cancer, justifying a prophylactic bilateral oophorectomy at the age of 40 years or before. Therefore, some oncologists do not recommend transplantation of cryopreserved ovarian tissue in these cases or to choose a site where close monitoring is feasible (Lambertini *et al.*, 2019). Another approach is to transplant the tissue only on the ovarian site and to perform bilateral oophorectomy as soon as patient has completed her family (Lambertini *et al.*, 2018).

Recommendation

Long-term risks in human are considered to be low but a long-term follow-up of patients after OTT is probably recommended	**GPP**
OTT can be offered in BRCA patients, but the ovarian tissue must be completely removed after subsequent pregnancy.	**WEAK ⊕○○○**

Justification

Data suggest that the procedure is safe. Malignant transformation of the grafted tissue has never been reported. However, malignant transformation has been described in animal studies after transplantation at heterotopic sites. Furthermore, clinical data are still scarce and possibly insufficient to make definite conclusions. As a safety precaution, long-term follow up of the patients and transplanted tissue is warranted.

Although there is no evidence for malignant transformation of or ovarian cancer originating from the grafted ovarian tissue, it seems safe to remove all the grafted tissue after the patients has completed her family, possibly in combination with prophylactic oophorectomy.

Research recommendations

- Evaluate the effectiveness of OTC in restoring fertility in larger cohorts of patients.
- Evaluate long-term safety of OTC and replacement for patients and their children (long-term follow-up).
- Develop highly sensitive methods for detection of neoplasic cells within the ovarian cortex of high-risk patients.

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770

D7 In vitro maturation (IVM)

In ART, in vitro maturation (IVM) is mainly used for women with polycystic ovary syndrome (PCOS) to avoid the risk of ovarian hyperstimulation syndrome (OHSS). IVM involves culture (for 24 to 48h) of immature cumulus-oocyte complexes (COCs) recovered from small antral follicles of patients that received no or mild FSH stimulation. Although the overall success rates with in vitro matured oocytes are lower compared to IVF, the births of over 5000 children (Sauerbrun-Cutler *et al.*, 2015) have been reported with no increase in congenital anomalies when compared to IVF children (Foix-L'Helias *et al.*, 2014, Roesner *et al.*, 2017, Mostinckx *et al.*, 2019)

In a fertility preservation programme, IVM can be offered as an alternative when conventional ovarian stimulation is contraindicated, or when the time available before the start of gonadotoxic treatment is short and cannot be delayed for ovarian stimulation treatment (Demirtas *et al.*, 2008). The key aspects of IVM that allow its use in these situations are that exogenous FSH administration may be avoided or minimally administered, and that oocytes can be retrieved independently of the phase of the menstrual cycle.

PICO Question: Should in vitro maturation be used for fertility preservation?

In vitro maturation (IVM) after in vivo oocyte aspiration

In IVM after in vivo oocyte aspiration, COCs are retrieved from the ovaries at germinal vesicle (GV) stage without previous exogenous gonadotropin administration followed by maturation to Metaphase II (MII) oocytes and vitrification or fertilization for embryo cryopreservation.

Hormonal priming

To improve the outcomes of IVM cycles, gonadotrophins can be administered before COC retrieval (called "priming"). Reports on IVM-FP protocols include the administration of hCG or GnRH agonist (GnRHa) 36h before oocyte pick-up, often but not always preceeded by a few days of FSH stimulation (Demirtas *et al.*, 2008, Maman *et al.*, 2011, Hourvitz *et al.*, 2015, Grynberg *et al.*, 2016, Sonigo *et al.*, 2016, Creux *et al.*, 2017, Creux *et al.*, 2018, El Hachem *et al.*, 2018, Kedem *et al.*, 2018). Priming protocols including low doses of FSH alone (37.5 – 150 IU/day for 3 to 6 days) have been largely used for infertile patients and, when not contraindicated, they might be used similarly in FP patients (De Vos *et al.*, 2011). Similarly, a 'pre-IVM' protocol involving initial treatment of oocytes with a meiosis inhibitor has been described and used clinically (Vuong *et al.*, 2020).

Only immature oocytes can be retrieved in patients unexposed to hCG or endogenous LH. However, upon hCG or GnRH agonist trigger, up to 20% of mature oocytes can be obtained at oocyte pick-up from which some can be recovered even from antral follicles of smaller diameter sizes (≤10 mm) (Nogueira *et al.*, 2012)

In a study comparing hCG and GnRHa solely as priming, a higher number of oocytes were retrieved with GnRHa priming (9.1 ± 6.8 versus 7.7 ± 5.5), but there was no difference in the total number of oocytes vitrified after IVM (El Hachem *et al.*, 2018).

In a comparison of IVM results according to the phase of the cycle (follicular or luteal phase) during which COC retrieval was performed after hCG trigger, there was no difference in the number of COCs recovered (9.3 ± 0.7 versus 11.1 ± 0.8), or the number of MII oocytes cryopreserved (6.2 ± 0.4 versus 6.8 ± 0.5) (Grynberg *et al.*, 2016). Similar results were reported in another study comparing outcomes after oocytes collected in the early follicular, late follicular, and luteal phases. This study reported no statistically significant differences in the number of oocytes collected (8.5 [4-15.8], 8 [5-14], and 7 [4-9], respectively), or the number of oocytes cryopreserved (3 [0-7.3], 3 [0-7], and 3 [1-5.5], respectively) (Creux *et al.*, 2017). Another small study also reported no difference in the outcomes of IVM after follicular versus luteal phase oocyte pick-up (Maman *et al.*, 2011).

Oocyte pick-up

For IVM, the methodology of oocyte pick-up differs from that used for conventional IVF since it is more abrupt involving concomitant aspiration and needle-forced detachment of granulosa cells from the wall of small antral follicles, thus increasing blood contamination of follicular fluid. General anaesthesia is generally used in IVM to facilitate the process of oocyte pick-up for practitioners and to provide more comfort to the patients. In view of these differences from conventional oocyte pick-up after ovarian stimulation, clinical and laboratory personnel need specific expertise to optimize oocyte pick-up, recovery and maturation rates.

Number of oocytes collected

The mean number of oocytes retrieved from IVM is generally less than would be expected after ovarian stimulation and has been reported to be between 5 and 17 in cancer patients (Moria *et al.*, 2011, Creux *et al.*, 2018).

A retrospective study by Creux and colleagues reported outcomes of 207 IVM procedures and 187 IVF procedures for FP in a mixed population of cancer patients. In breast cancer patients, IVM was more often performed (72.4%) mainly because of concerns regarding ovarian stimulation in women with hormone-dependent cancers, while patients with haematological or other cancer more often received IVF treatment. There was a significantly higher number of oocytes collected with IVF (12 [8–18] versus 7 [5–12.5]), and higher number of MII oocytes (9 [5–12] versus 2 [1–3]). The study further reported a higher number of oocytes (10 [6–15] versus 5 [2–8]) and embryos cryopreserved (5 [3–7] versus 3 [2–5]) (Creux *et al.*, 2018). Moria and colleagues compared the outcomes of IVM (with hCG priming) in women with different types of cancer with a control group of infertile women. The number of retrieved oocytes was significantly lower in breast cancer patients (but not the other patient groups) compared to the control group (9[6-16] versus 12[7-20]). There was no difference in the percentage of in vivo matured oocytes or the percentage of MII oocytes that matured in vitro (Moria *et al.*, 2011).

The latter study showed oocyte maturation rates in cancer patients ranging from 50 to 61.2%, with no difference between the patient groups (Moria *et al.*, 2011).

A study by Grynberg and colleagues retrospectively reviewed outcomes of IVM procedures in women with BRCA-positive and BRCA-negative breast cancer patients and reported no difference in the number of COCs retrieved (8.9 ± 6.9 versus 9.9 ± 8.1 oocytes), IVM rates (67 ± 24 versus 62 ± 23%) and the number of MII oocytes cryopreserved (5.1 ± 3.8 versus 6.1 ± 5.1, respectively) (Grynberg *et al.*, 2019).

Prediction of the number of oocytes collected and cryopreserved after IVM

Correlation between antral follicle count (AFC) or AMH levels with the number of collected oocytes and the number of matured oocytes cryopreserved has been performed (Sonigo *et al.*, 2016). In a retrospective analysis of 300 patients with breast cancer, they showed that in patients with AMH levels on day 3 of ≥ 3.5 ng/ml and with AFC ≥ 19, 8 or more matured oocytes could be cryopreserved (Sonigo *et al.*, 2016). Similarly, Sermondade and colleagues reported a moderate positive correlation between AMH levels and AFC with the number of recovered COCs in breast cancer patients, and with the number of matured oocytes after IVM (Sermondade *et al.*, 2019).

The study by Grynberg mentioned previously also measured AFC and AMH levels, and reported no difference in these parameters, nor in IVM rates and number of cryopreserved oocytes in breast cancer patients with or without BRCA mutations (Grynberg *et al.*, 2019).

Cryopreservation of IVM oocytes

Oocytes can be cryopreserved at either the immature germinal vesicle (GV) or the mature MII stage, meaning either before or after IVM. Regarding the feasibility of the former option, there are limited data on oocyte capability for maturation and survival after vitrification. Furthermore, currently available data mostly involve surplus GV oocytes retrieved after conventional ovarian stimulation, of which the inherent quality may differ from GV oocytes retrieved in IVM cycles. The results

indicate that maturation rates of GV-stage oocytes are higher when IVM is performed before vitrification than after (Kasapi *et al.*, 2017). Until a protocol to protect cumulus-enclosed oocytes for vitrification is developed, the consensus is to vitrify oocytes at the mature state, i.e. metaphase II (Combelles and Chateau, 2012).

Live births following IVM

Following improvements in the technique of oocyte vitrification throughout the last decade, about 10 live births have been reported from embryos derived from vitrified IVM oocytes from infertile patients. Among those reported births, the largest series of patients (n=20) led to 4 live births (Chian *et al.*, 2009). Given this paucity of data in the infertile population, it is not surprising that there is even less data in the context of FP. In a series of women with cancer who cryopreserved oocytes after IVM (total of 207 IVM cycles), 6.5% returned to use them (Creux *et al.*, 2018). Three live births have been reported in cancer patients, from which two were after IVM and embryo vitrification (Creux *et al.*, 2018) (Kedem *et al.*, 2018), and one recently reported from oocytes vitrified after IVM (Grynberg *et al.*, 2020).

In vitro maturation (IVM) after ex vivo extraction of oocytes from ovarian specimens

In order to maximize the fertility preservation potential in patients where ovarian tissue is being surgically removed, it can be possible to recover immature oocytes from within ovariectomy specimens during tissue processing for cryopreservation. This strategy can be useful where ovariectomy is part of the treatment of cure (i.e. in ovarian cancer) or when ovarian tissue is being processed for cryopreservation. Since the first report of two cases (Isachenko *et al.*, 2004), several small and moderate-sized case series have shown the feasibility of this technique in prepubertal and adult women. Studies containing at least 25 oncological and non-oncological patients aged from 0 - 44 years old resulted in oocyte recovery from 87% of ovarian tissue specimens, with a range of 0 to 58 oocytes recovered, with mean values 14.7 ± 2.2 (Segers *et al.*, 2015), 10.9 ± 9.4 (Yin *et al.*, 2016), and 11.2 ± 7.9 (Wilken-Jensen *et al.*, 2014). A study involving only breast cancer patients reported a mean of 8.3 ± 6.1 (range: 0 – 26) oocytes recovered (Takae *et al.*, 2015). Lower oocyte recovery rates were reported in patients who had previously received chemotherapy (7 vs 12), in patients aged 2–18 years old (Abir *et al.*, 2016), but the safety of the procedure and the quality of the retrieved oocytes is questionable.

In a series of 255 cancer patients, performing IVM aspiration prior to ovarian tissue harvesting in addition to ex vivo oocyte extraction was reported to increase the yield of immature oocytes (11.87 ± 1.22 versus 6.95 ± 0.83) and oocytes cryopreserved (6.45 ± 0.81 versus 2.47 ± 0.41) (Hourvitz *et al.*, 2015).

Reported oocyte maturation rates after 48h culture vary from 23 to 62% from studies involving more than 25 patients (Segers *et al.*, 2015, Takae *et al.*, 2015, Yin *et al.*, 2016, Fasano *et al.*, 2017, Kedem *et al.*, 2018). A case involving a mosaic Turner syndrome patient reported the recovery of 11 immature oocytes with 8 (73%) of them becoming matured and were vitrified (Huang *et al.*, 2008).In general, it seems that survival rate and maturation capacity of *ex vivo* extracted oocytes from ovarian tissue may be lower than that of in vivo aspirated oocytes (Kedem *et al.*, 2018).

Transportation of samples

Prolonged exposure to low temperatures during transportation (Isachenko *et al.*, 2009, Shirasawa *et al.*, 2019), the condition of specimens (whether intact ovary or biopsy), and the fact that immature oocytes are collected from non-selected antral follicles including those of very small sizes (< 6 mm) might account for the generally lower maturation rates of ex vivo extracted oocytes. No human studies have been performed to define the optimal protocol to keep both ovarian follicles and oocytes within ovarian tissue in a healthy state during transportation; it is likely that the cooled temperatures needed for tissue transport (where the primary objective is survival of primordial

follicles and stroma) is significantly detrimental to the developmental competence of subsequently extracted oocytes from antral follicles.

Live births following IVM

High fertilization rates can be obtained in *ex vivo* matured oocytes (≥ 65%), however, data are limited and absent for vitrified-warmed oocytes (Segers *et al.*, 2015). Insufficient data are available on the efficacy of vitrification for oocyte and embryo survival after warming. To date, a total of three cases of healthy live birth have been reported following transfer of vitrified embryos derived from *ex vivo* IVM oocytes: from a 21-year old patient with ovarian carcinoma (Prasath *et al.*, 2014), a 23-year-old patient with borderline ovarian tumour (Uzelac *et al.*, 2015) and a 26-year-old with a benign condition (Segers *et al.*, 2015). In all three reports, the ovaries were cooled to 4˚C for a period of 20 min to 3 hours.

Recommendations

IVM should be regarded as an innovative FP procedure.	**STRONG** ⊕○○○
IVM requires specific expertise and should only be performed when oocyte cryopreservation is required but ovarian stimulation not feasible.	**GPP**
IVM after ex vivo extraction is considered an experimental procedure	**WEAK** ⊕○○○

Justification

Data on the efficacy of IVM technique for fertility preservation are limited to rates of oocyte recovery and maturation. Few data are available on subsequent fertilization and embryo implantation.

With data of proof-of-principle, but in absence of long-term safety data, procedural reliability and high effectiveness, the technique is to be categorized as "innovative" (rather than established) (Provoost et al., 2014). Recommendations for innovative treatments should be followed, including monitoring of data and informing patients. The GDG highlighted one of these recommendations - only centres with expertise about the procedure should offer innovative treatment to their patients- in the second recommendation.

IVM after ex vivo extraction is considered an experimental treatment, based on the same categorization of treatments, but with even more uncertainties. As such, centres offering IVM after ex vivo extraction should do so only after approval by a medical research ethics committee.

Recommendations for research

More studies are needed on the quality of the oocytes after IVM and the long-term outcomes (epigenetic factors etc)

The protocols used for IVM should be further standardized to ensure the technique is reliable. Aspects to be considered in this are the timings and whether cryopreservation should be done before or after IVM.

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D8. GnRH agonists

In premenopausal women undergoing chemotherapy for malignant or benign diseases, besides the risk of infertility, treatment-induced premature ovarian insufficiency (POI) can lead to several other short- and long-term negative consequences on their quality of life and wellbeing (Webber *et al.*, 2016). In this setting, concurrent administration of GnRH agonists has been widely studied as a strategy for ovarian protection to reduce the risk of treatment-related POI.

Despite preclinical experiments generally supporting the efficacy of this option, it should be highlighted that the mechanism of action for the ovarian protective effect of GnRH agonists use during chemotherapy remains not fully clarified (Lambertini *et al.*, 2019). Nevertheless, in the clinical setting, several randomized trials have provided evidence on the efficacy and safety of administering GnRH agonists for ovarian protection during chemotherapy, the majority relating to women with breast cancer with more limited evidence in those with other solid tumours, haematological malignancies or benign diseases. Notably, variable definitions of treatment-related POI and timepoints of its evaluation following the end of chemotherapy have been used in the different studies. In the majority, amenorrhoea alone was considered to define treatment-induced POI; however, some studies used a composite endpoint for its definition (i.e. amenorrhoea and post-menopausal hormonal levels) as currently recommended by guidelines (Webber *et al.*, 2016). Additionally, there are very few data on ovarian function beyond 2 years after chemotherapy, and none of the studies that assessed the efficacy and safety of this strategy aimed primarily to investigate its fertility preservation potential. Only a minority of them have adequate follow-up to report on post-treatment pregnancies; patients' wish to conceive was not an inclusion criterion nor was this information systematically collected during any of these studies.

PICO QUESTION: SHOULD GNRH AGONISTS VS. NO TREATMENT BE USED FOR OVARIAN PROTECTION IN PATIENTS UNDERGOING GONADOTOXIC TREATMENT?

Cancer

Breast cancer

A total of 14 randomized trials have been conducted to investigate the efficacy and safety of administering GnRH agonists during chemotherapy as a strategy for ovarian protection in premenopausal women with early-stage breast cancer (Lambertini *et al.*, 2019). The efficacy data from 5 major trials were summarized in a meta-analysis based on individual patient-level data from 873 premenopausal breast cancer patients (Lambertini *et al.*, 2018). Median age was approximately 38 years. In women who received chemotherapy with or without GnRH agonist, chemotherapy-induced POI rates were 14.1% and 30.9%, respectively (adjusted odds ratio [OR] 0.38; 95% CI 0.26-0.57). The ovarian protective effect of GnRH agonists was observed irrespective of patients' age at the time of treatment, estrogen receptor status, type and duration of chemotherapy. In terms of FP potential, 37 of 359 women treated with GnRH agonists during chemotherapy had at least one post-treatment pregnancy compared to 20 of 367 women treated with chemotherapy alone (incidence rate ratio [IRR] 1.83; 95% CI 1.06-3.15) (Lambertini *et al.*, 2018). In the POEMS/SWOG S0230 trial (i.e. the only study with post-treatment pregnancies as pre-planned secondary endpoint), the 5-year cumulative incidence of pregnancy was significantly higher in the chemotherapy plus GnRH agonist arm as compared to the chemotherapy alone arm (23.1% vs. 12.2%; OR 2.34; 95% CI 1.07-5.11) (Moore *et al.*, 2019). Among the several available meta-analyses based on abstracted data, the largest one which included 1,231 premenopausal breast cancer patients from 12 trials showed similar results with significant reduction in chemotherapy-induced POI rates and increased pregnancy rates in patients who received concurrent GnRH agonists during chemotherapy (Lambertini *et al.*, 2015).

In the few trials that assessed the actual protective effect of administering GnRH agonists during chemotherapy on patients' ovarian reserve, no difference was observed in the levels of anti-Müllerian hormone (AMH) before and after treatment between treatment arms (Lambertini *et al.*, 2019). However, within these trials, AMH levels were available only for a minority of the randomized patients; the largest analysis was conducted in the Anglo Celtic Group OPTION trial with AMH data available for approximately half of the study population (Leonard *et al.*, 2017). A potential protective effect on patients' ovarian reserve was observed in a prospective cohort study including 88 premenopausal women with newly diagnosed breast cancer; antral follicle count recovered faster and to a greater degree for those who received GnRH agonists during chemotherapy (Sinha *et al.*, 2018).

Regarding safety, the administration of GnRH agonists is associated with significant higher rates of hot flushes and sweating (Lambertini *et al.*, 2018). Bone turnover is increased during administration of GnRH agonists with normalization after cessation of treatment and with the potential to protect against longstanding altered bone turnover associated with POI (Wilson *et al.*, 2016). In premenopausal breast cancer patients and particularly in those with estrogen receptor-positive disease, there are potential safety concerns regarding possible antagonism between GnRH agonists and chemotherapy. However, no difference in disease-free survival (hazard ratio [HR] 1.01; 95% CI 0.72-1.42) and a non-significant trend towards better overall survival (HR 0.67; 95% CI 0.42-1.06) with concurrent use of GnRH agonists during chemotherapy was observed in the individual patient-level data meta-analysis; no interaction according to estrogen receptor status was found (Lambertini *et al.*, 2018). The lack of detrimental effect on survival outcomes with concurrent administration of GnRH agonists during chemotherapy was also confirmed in two large adjuvant endocrine therapy trials in premenopausal breast cancer patients with estrogen receptor-positive disease (Regan *et al.*, 2017).

Recommendations

GnRH agonists during chemotherapy should be offered as an option for ovarian function protection in premenopausal breast cancer patients receiving chemotherapy; however, limited evidence exists on their protective effect on the ovarian reserve and the potential for future pregnancies.	**STRONG**	⊕⊕⊕⊕

In women with breast cancer, GnRH agonists during chemotherapy should not be considered an option for fertility preservation instead of cryopreservation techniques.	**STRONG**	⊕⊕⊕◯

Justification

Trials on ovarian function protection in premenopausal breast cancer patients have been summarized in several systematic reviews and meta-analyses, and all the most recent ones including the majority of the trials showed similar and consistent conclusions. A recent analysis based on individual patient-level data of 873 premenopausal breast cancer patients was considered the highest quality of evidence (Lambertini et al., 2018). Concurrent administration of GnRH agonists and chemotherapy significantly reduced the risk of developing chemotherapy-induced POI, was associated with a higher number of post-treatment pregnancies and had no negative impact on survival outcomes. Main adverse events associated with GnRHa administration are vasomotor symptoms and sexual problems. Overall, ovarian protection with GnRH agonists is feasible and acceptable in this setting.

Trials in premenopausal breast cancer patients mainly investigated the impact on chemotherapy-induced POI with only one trial having post-treatment pregnancies as pre-planned secondary endpoint. Use of GnRH agonists during chemotherapy seems to increase the chances of post-chemotherapy pregnancies but data on this regard are less abundant. Given the limited data on post-treatment pregnancies, the GDG stresses that GnRH agonists should not replace oocyte or embryo cryopreservation in patients interested in fertility preservation. In this setting, ovarian protection can be

used in addition to oocyte or embryo cryopreservation, or as a single FP option where oocyte or embryo cryopreservation is not feasible.

Malignancies other than breast cancer

The available evidence on the efficacy and safety of administering GnRH agonists during chemotherapy as a strategy for ovarian protection in premenopausal patients with malignancies other than breast cancer is limited. Four small, randomized trials were conducted in women with haematological malignancies and one in patients with ovarian cancer (Lambertini *et al.*, 2019).

Lymphoma

Among the several available meta-analyses based on abstracted data, the largest one that summarized the results from 3 randomized trials conducted in women with haematological malignancies included 109 patients with Hodgkin and non-Hodgkin lymphoma (Senra *et al.*, 2018). Median age was approximately 25 years; patients received chemotherapy regimens with different gonadotoxicity ranging from low (e.g. ABVD [doxorubicin, bleomycin, vinblastine and dacarbazine] protocols) to high (e.g. conditioning regimens for haematopoietic stem cell transplantation). No significant difference in POI rates was observed between lymphoma patients who received chemotherapy with or without concurrent GnRH agonists (18.9% vs. 32.1%; OR 0.70, 95% CI 0.20-2.47). Few post-treatment pregnancies (17 vs. 18) were described without difference between patients who received GnRH agonists during chemotherapy or not (OR 1.13, 95% CI 0.66-1.93)(Senra *et al.*, 2018). The largest randomized trial included in the meta-analysis reported on AMH levels before and after treatment (Demeestere *et al.*, 2016), although of 84 patients included, 37 had AMH levels available at least once during study follow-up. Significantly higher AMH levels were observed in patients who received GnRH agonists during chemotherapy at one year follow-up, but not at later follow-up (2-4 and 5-7 years) (Demeestere *et al.*, 2016).

. An additional medical benefit of administering GnRH agonists during chemotherapy is prevention of heavy menstrual bleeding which may be of value for patients receiving chemotherapy regimens with high bone marrow toxicity.

Observational data suggest that oral contraceptives may also reduce the risk of POI, but no propper randomized studies have demonstrated their effect (Behringer *et al.*, 2005) .

Ovarian cancer

One randomized trial reported on the use of GnRH agonist treatment in 30 premenopausal women with ovarian cancer receiving cyclophosphamide- and platinum-based chemotherapy regimens (Gilani *et al.*, 2007). Six months after chemotherapy, all the patients who received GnRH agonists during chemotherapy had normal menstrual bleeding, while 33% of those treated with systemic cytotoxic therapy alone had treatment-induced POI. No information on post-treatment pregnancies was available.

Recommendation

In malignancies other than breast cancer, GnRH agonists should not be offered as an option for ovarian function protection and fertility preservation.	**STRONG** ⊕○○○

Justification

Data for malignancies other than breast cancer are limited and available only for patients with lymphoma or ovarian cancer. For lymphoma, evidence on ovarian function protection and post-treatment pregnancies is limited with no clear difference between patients receiving GnRH agonist treatment or not. For ovarian cancer patients, the only small available trial showed a potential effect in terms of ovarian function protection but did not report on fertility outcomes.

Given the lack of solid data on its efficacy, GnRH agonist treatment should not be offered for ovarian function protection and fertility preservation to patients undergoing gonadotoxic treatment for malignancies other than breast cancer.

Benign diseases

The efficacy and safety of administering GnRH agonists as a strategy for ovarian protection have been investigated in several (mostly non-randomized) studies of premenopausal women with autoimmune diseases receiving cyclophosphamide.

A meta-analysis including four prospective cohort studies in 83 patients with systemic lupus erythematosus (SLE) receiving cyclophosphamide showed that concurrent administration of GnRH agonists was associated with a significant reduction in risk of developing treatment-induced POI (OR 0.12; 95% CI 0.03-0.41) (Ben-Aharon *et al.*, 2010). Limited data on post-treatment pregnancies were reported: a total of 13 and 3 post-treatment pregnancies were described in women who received chemotherapy with or without GnRH agonists, respectively (Ben-Aharon *et al.*, 2010). A randomized, placebo-controlled, dose-escalation trial in 31 premenopausal patients with SLE receiving cyclophosphamide was conducted to assess the optimal dose of the GnRH agonist triptorelin for obtaining complete ovarian suppression (Brunner *et al.*, 2015). A weight-adjusted dose of 120µg/kg body weight provided sustained complete ovarian suppression in 90% of the patients without increased risk of adverse events (Brunner *et al.*, 2015). In a retrospective biomarker analysis conducted within a prospective cohort study, AMH levels before and after treatment were compared between premenopausal patients with SLE receiving cyclophosphamide alone (n=11) or with concurrent GnRH agonists (n=10) (Marder *et al.*, 2012). Higher post-treatment AMH levels were observed in patients receiving GnRH agonists during cyclophosphamide (Marder *et al.*, 2012).

Recommendation

GnRH agonists during chemotherapy may be considered as an option for ovarian function protection in premenopausal patients with autoimmune diseases receiving cyclophosphamide. However, it should be acknowledged that limited data are available in this setting.	**WEAK** ⊕⊕◯◯

Justification

Data supporting this recommendation include one randomized trial and 3 prospective and retrospective studies summarized in a meta-analysis of a total of 83 patients with SLE. Overall, there seems to be some benefit of GnRH agonist treatment concurrent with cyclophosphamide.

With regards to safety, there are no apparent safety issues regarding the use of GnRH agonists, while standard fertility preservation procedures could confer an increased risk of important adverse events in patients with severe vasculitis. Weighing the possible benefits and risks in this specific patient group, GnRH agonists during chemotherapy may be considered as an option for ovarian function protection.

GnRH agonists for Fertility Preservation (all patients)

General recommendation

GnRH agonists should not be considered an equivalent or alternative option for fertility preservation but can be offered after cryopreservation techniques or when they are not possible.	**GPP**

Justification

In general, evidence seems to show some benefit of concurrent GnRH agonists during chemotherapy for preserving fertility in breast cancer patients, although the benefit was not observed in women with

other diseases. The reasons for this discrepancy are probably dependent on different methodological and clinical factors but are not fully established. GnRH agonists are generally considered a safe and feasible option.

The GDG considered that GnRH agonists should not be offered as a single FP option. Therefore, the GDG formulated a good practice point against GnRH agonists protection as a single FP option, unless other FP options are not possible.

Research recommendations

Research efforts are needed to provide more evidence on the role of GnRH agonists in ovarian function protection for patients with diseases other than breast cancer. In addition, the collection of long-term follow-up data (including pregnancies and age at menopause) from the already existing randomized trials should be encouraged to provide more robust evidence on the role of this strategy also for fertility preservation. Finally, well-designed and adequately conducted *in vitro* and *in vivo* experimental studies should be conducted also in species other than rodents to finally elucidate the protective mechanisms of action of this strategy.

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D9. Ovarian transposition

Radiation therapy is indicated for treatment of pelvic malignancies including cancers of the cervix, endometrium, rectum, bladder, as well as sarcomas and lymphomas that involve the pelvic region. The radiation dose applied in the treatments is ranging from a minimum of 30 Gray (Gy) in divided doses for treatment of lymphoma, to as high as 60 Gy for local treatment of advanced cancers.

The ovaries are highly radiosensitive and the cutoff dose for radiation-induced ovarian failure is age-dependent. Wallace and colleagues estimated, using mathematical models, that the required dose of fractionated radiotherapy to induce ovarian failure is higher in younger girls and decreases with age, being 18.4 Gy at 10 years, 16.5 Gy at 20 years and 14.3 Gy at 30 years (Wallace *et al.*, 2005).

To protect the ovaries from the direct negative effects of radiation, surgical ovarian transposition (OT) (i.e. surgical repositioning of ovaries away from the radiation) before initiation of radiotherapy, has been proposed and a number of observational studies of ovarian transposition are available. The studies include patients within a wide range of age from 11 to over 40 years, heterogeneous diagnoses and different types of radiotherapy, including both external beam radiotherapy and brachytherapy, although the vast majority of patients reported in OT studies have been treated for cervix cancer. There is a high variation in how ovarian function was evaluated after surgery, mostly after short-term follow-up. Depending on the radiotherapy field planned, two surgical techniques for OT have been described, using either lateral or medial transposition approaches. In recent years, less invasive surgical techniques, such as laparoscopy and robot-assisted laparoscopy, have been reported in OT.

In general, the procedure of OT has demonstrated feasibility, and according to some authors it is underused (Gubbala *et al.*, 2014).

PICO Question: Should Transposition of ovaries versus no treatment be used for ovarian protection?

Efficacy

Preservation of ovarian function

Studies of ovarian transposition have all been observational, mostly small and retrospective including cases and case series reports and most of the studies have been uncontrolled.

A systematic review and meta-analysis evaluated ovarian function and risks of OT using data from studies published up to 2014 (Gubbala *et al.*, 2014). The authors included 24 observational studies, of which 7 were prospective in their meta-analysis, with a total of 892 women who underwent OT before radiotherapy for treatment of cancer, mostly cervical cancer (n=828). Half of the women (n=428) underwent radical surgery including hysterectomy without radiotherapy, 143 had post-operative brachytherapy and 321 had post-operative external-beam radiotherapy. Preservation of ovarian function was found in 90% (95% CI 82–99), 90% (95% CI 79–111) and 56% (95% CI 56–74) of women in each of the groups, respectively, calculated from data (available from approximately 50% of the total number of women included) of ovarian function (either symptoms or serum FSH levels). The mean follow-up was longer than 12 months in 79% of the studies (Gubbala *et al.*, 2014).

A more recent review included 38 studies; however a meta-analysis was not performed due to heterogeneity among the studies (Hoekman *et al.*, 2019). Successful preservation of ovarian function after ovarian transposition and external-beam radiotherapy (with or without brachytherapy) ranged from 20 to 100% (26 studies n = 401), after a median follow-up time ranging from 7 to 102 months. A higher frequency of preserved ovarian function was found in women who received brachytherapy only, from 63.6 to 100% (8 studies, n=148). In patients who received radiation therapy and chemotherapy, preservation of ovarian function ranged from 0 to 69.2% (5 studies, n=81).

Impact of age

Retrospective data indicate that older women may have a lower probability of preserving ovarian function after ovarian transposition. In the study of Hoekman and colleagues, 27 women with cervical cancer treated with hysterectomy/trachelectomy and radiation therapy underwent ovarian transposition and 29 women receiving similar cancer treatment were included as controls (Hoekman *et al.*, 2018). Ovarian failure was defined as climacteric complaints (with or without starting hormone replacement therapy) and/or laboratory measurements (FSH >40 IU/L and/or estradiol <100 pmol/L), or bilateral salpingo-oophorectomy. The authors reported the 5-year rate for ongoing ovarian function (ovarian survival), with a sub-analysis for age (25-30, 31-35 and 36-40 years). The radiation dose was 44.8Gy (25.0-63.0Gy) and 46.3Gy (45.0-50.0Gy), respectively in patients with and without transposed ovaries. The 5-year ovarian survival rate was 60.3 in women that had ovarian transposition versus 0% in controls (95% CI 3.48-11.50). There was a decrease in ovarian survival with increasing age, nevertheless, ovarian survival was significantly higher after ovarian transposition in all age groups compared to controls. No conclusions could be made on women older than 40 years due to loss of follow-up (Hoekman *et al.*, 2018).

Impact of ovarian transposition on sex hormone levels

The impact of ovarian transposition on sex hormone levels (estradiol [E_2], progesterone, FSH, LH) was assessed in a study - published after the Gubbala meta-analysis - of 86 women with cervical cancer of whom 13 underwent ovarian transposition of one ovary and 73 of both ovaries (Du and Qu, 2017). Patients undergoing different radiotherapy treatments were compared with a control group that did not receive radiotherapy. In the latter group, there were no differences in the sex hormone levels measured at different timepoints, while in the patients who received radiotherapy, sex hormone levels were significantly different after as compared to before radiotherapy, indicating that OT did not prevent the effect of radiotherapy (Du and Qu, 2017). Another similar study evaluated sex hormone levels (E_2, FSH) and menopausal symptoms in 105 patients undergoing intensity-modulated radiotherapy (IMRT) with a limited radiation dose to the ovaries; 48 of these patients received unilateral ovary transposition, while 57 received bilateral transposition. Preservation of ovarian function was found in 41 patients (39.0%) when a low radiation dose was received, regardless of bilateral or unilateral involvement of the ovaries (Yin *et al.*, 2019).

Pregnancy after ovarian transposition

Several pregnancies have been reported after ovarian transposition, including natural conceptions (Morice *et al.*, 1998, Terenziani *et al.*, 2009), as well as pregnancies after IVF with transabdominal oocyte collection (Jang *et al.*, 2019) and surrogacy (Selvaraj *et al.*, 2019). An observational study included 27 women with cervical cancer (treated with surgery, bilateral ovarian transposition and radiotherapy) and 10 women with ovarian dysgerminoma (treated with surgery, unilateral ovarian transposition and radiotherapy)(Morice *et al.*, 1998). and reported pregnancy rates of 15% (4/27) and 80% (8/10) in the 2 study groups, respectively. Three women underwent repositioning of the ovaries after persistent infertility, with pregnancy achieved in one of them. The median time interval between the end of tumour treatment and the first conception was 4.3 years (range 2-7 years). Of the 18 pregnancies, five ended in a miscarriage (5/18; 28%) and 13 successful pregnancies produced 15 liveborn children (Morice *et al.*, 1998).

Complications

In the systematic review by Hoekman, a total of 112 (12.8%) complications were identified in 872 patients after ovarian transposition (22 studies). Complications consisted of ovarian cyst development (93/112; 83.0%), abdominal pain (6/112; 5.4%), haematoma (2/112; 1.8%), tubal ligation (1/112; 0.9%), ischemia (1/112; 0.9%), and unspecified complications (2/112; 1.8%). Reoperation (for various reasons, not specified in 26 patients) was necessary in 40 of 112 complications (34.7%). Ovarian metastasis was found in 5 patients (0.9%) treated for cervical cancer from a total of 538 patients with that diagnosis (Hoekman *et al.*, 2019).

Cyst development and ovarian metastasis were also reported in the meta-analysis by Gubbala, with cysts found in 13% of the transposed ovaries. The reviewers suggested a higher incidence of cysts in patients who underwent subcutaneous versus lateral ovarian transposition (Gubbala *et al.*, 2014). Ovarian cancers or metastasis were not found (Gubbala *et al.*, 2014).

Another complication reported, not included in the meta-analyses (which excluded case reports), is ovarian torsion, which can occur (Gomez-Hidalgo *et al.*, 2015).

Technical considerations

Medial transposition vs lateral transposition.

Depending on the planned radiotherapy field, two surgical techniques have been described, lateral and medial transposition approaches (Moawad *et al.*, 2017). In small controlled studies, lateral transposition is associated with a higher rate of preservation of ovarian function (Grabenbauer *et al.*, 1991, Moawad *et al.*, 2017).

Comparison single unilateral transposition versus bilateral transposition

A single unilateral transposition has been proposed as similarly successful to bilateral transposition, as supported by a small prospective study of 20 women (Clough et al., 1996). In that study ovarian function was maintained in up to 85% of cases. At present, there are no studies comparing unilateral versus bilateral transposition.

Additional surgery considerations and concomitant salpingectomy

Several authors have recommended salpingectomy concomitantly with the transposition surgery, to allow microscopic investigation of occult cancer in the tube (Huang *et al.*, 2007, Terenziani *et al.*, 2009). It is also recommended that surgical clips should be placed to identify the position of the ovaries.

Recommendations

Where pelvic radiotherapy without chemotherapy is planned, women may be offered ovarian transposition with the aim to prevent premature ovarian insufficiency	**WEAK** ⊕⊕◯◯

Women with reduced ovarian reserve and women at risk of having ovarian metastases are inappropriate candidates for ovarian transposition.	**GPP**

Justification

In general, a lack of well-designed clinical trials limits the quality of available data on the efficacy and safety of ovarian transposition. Studies of ovarian transposition have all been observational, mostly small and retrospective including cases and case series reports and most of the studies have been uncontrolled.

Current meta-analysis of observational data indicate that the procedure of ovarian transposition is feasible. Data also show that the procedure is efficacious with regards to ovarian function preservation (in most patients) and pregnancies have been reported. A single ovarian transposition seems to be sufficient to maintain ovarian function; however reproductive outcomes are seldom reported in a whole cohort and mostly as retrospective case series.

Regarding safety, an overall complication rate of 12.8% has been reported, mainly ovarian cysts not requiring additional intervention or treatment. In recent years, less invasive laparoscopy and robot-assisted laparoscopy have been applied to OT procedures and more information should be available in the future regarding preferred surgical techniques. At the moment, the OT technique is not standardized.

Recent data show that the efficacy of transposition to protect ovarian function is dependent on patient characteristics. In women of high reproductive age and/or with reduced ovarian reserve the benefits of the procedure may be smaller and not proportionate to the risks. Similarly, in women at risk of developing ovarian metastases, the procedure should not be recommended. The GDG therefore advises against ovarian transposition for these subgroups of patients.

The recommendation to offer ovarian transposition to women scheduled to undergo pelvic radiotherapy is in line with recommendations from the American society of Clinical Oncology (Lee et al., 2006, Oktay et al., 2018) and the National comprehensive Cancer Network recommend offering ovarian transposition as a fertility preservation option in patients with cancer (Koh et al., 2019).

Research recommendation

Well-designed clinical trials on the efficacy and safety of ovarian transposition are lacking

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PART E: After treatment care

With increasing survival rate after cancer, it is becoming more and more common for cancer survivors to get pregnant. Rates of pregnancy among cancer survivors are generally lower than age-matched peers but pregnancy does not appear to increase the risk of cancer recurrence (Duffy and Allen, 2009). However, there has been considerable concern and debate regarding the safety of pregnancy in women with cancer, and specifically with hormone-sensitive tumours such as breast cancer. Cancer patients also report concerns regarding the risks of chemotherapy to their offspring and the safety of pregnancy itself.

E1. Patient assessment prior to use of stored material

The increasing number of cancer survivors makes the issues of ovarian dysfunction and childbearing ability more and more relevant for the quality of life of these patients. For those who wish to start or increase their family after cancer, it is important to assess their reproductive function and potential for conception and successful pregnancy. This chapter will summarize how to re-assess reproductive function before use of stored material and/or in view of reproduction.

Narrative Question: How should patients be re-assessed before use of stored material?

Cancer patients

Ovarian damage

In a literature review of breast cancer survivors, the risk of ovarian failure in women under the age of 40 years was between 22-61%, whereas in women above the age of 40 years the risk was increased to 61-97% (Dabrosin, 2015). Most women who resume ovarian function after chemotherapy tend to get return of menses within 1 year, although menstrual irregularities are common (Goldman and O'Hair, 2009).

It is possible to assess the ovarian reserve before and after cancer treatment by performing serum anti-Müllerian hormone (AMH) assessments. It is known that AMH is lower in breast cancer survivors than in controls (Anderson *et al.*, 2006, Lutchman Singh *et al.*, 2007, Partridge *et al.*, 2010, Su *et al.*, 2010); AMH is lower after chemotherapy than before treatment (Anderson *et al.*, 2006, Lutchman Singh *et al.*, 2007, Anders *et al.*, 2008, Rosendahl *et al.*, 2010, Yu *et al.*, 2010, Henry *et al.*, 2014); after chemotherapy for breast cancer, AMH is higher in menstruating women than in patients who are amenorrhoeic (Anders *et al.*, 2008, Su *et al.*, 2010, Anderson and Cameron, 2011); and pre-treatment AMH is predictive of ovarian function, as based on menstrual function (Anderson *et al.*, 2006, Rosendahl *et al.*, 2010, Henry *et al.*, 2014).

However, it is important to recognize the limitations of AMH as a predictor of pregnancy, either though natural conception or after ART (Hagen *et al.*, 2012, Steiner *et al.*, 2017). There are very limited data on the relation between post-cancer AMH levels and pregnancy, but it is clear than even very low AMH levels do not preclude the chance of natural conception in the short-term (Hamy *et al.*, 2016, Anderson *et al.*, 2018).

Measurement of AMH after cancer may be of value in predicting remaining reproductive lifespan, i.e. time to menopause. There are no data directly assessing this in cancer survivors, but in healthy women, AMH has some predictive value. However, the added value over age is poor, particularly for prediction of early menopause (Depmann *et al.*, 2018). Assessment of the rate of decline through serial measurement is also uninformative (de Kat *et al.*, 2019) and seems to underestimate the risk

of early menopause. Recent data from women already approaching a natural menopause indicate that in that age group, a very low AMH can be an accurate predictor of the likelihood of having the final menstrual period within the next 12 months (Finkelstein *et al.*, 2020).

Uterine damage

Women who have been treated with radiotherapy to a field that includes the uterus have increased risks of pregnancy complications (see section E1. Patient assessment prior to use of stored material). This is an important risk factor to take into account in patient assessment and counselling. Measurement of uterine volume or function (e.g. uterine artery blood flow) may be of value, but prospective studies assessing their predictive performance have not been performed.

Assessment of infertility or POI

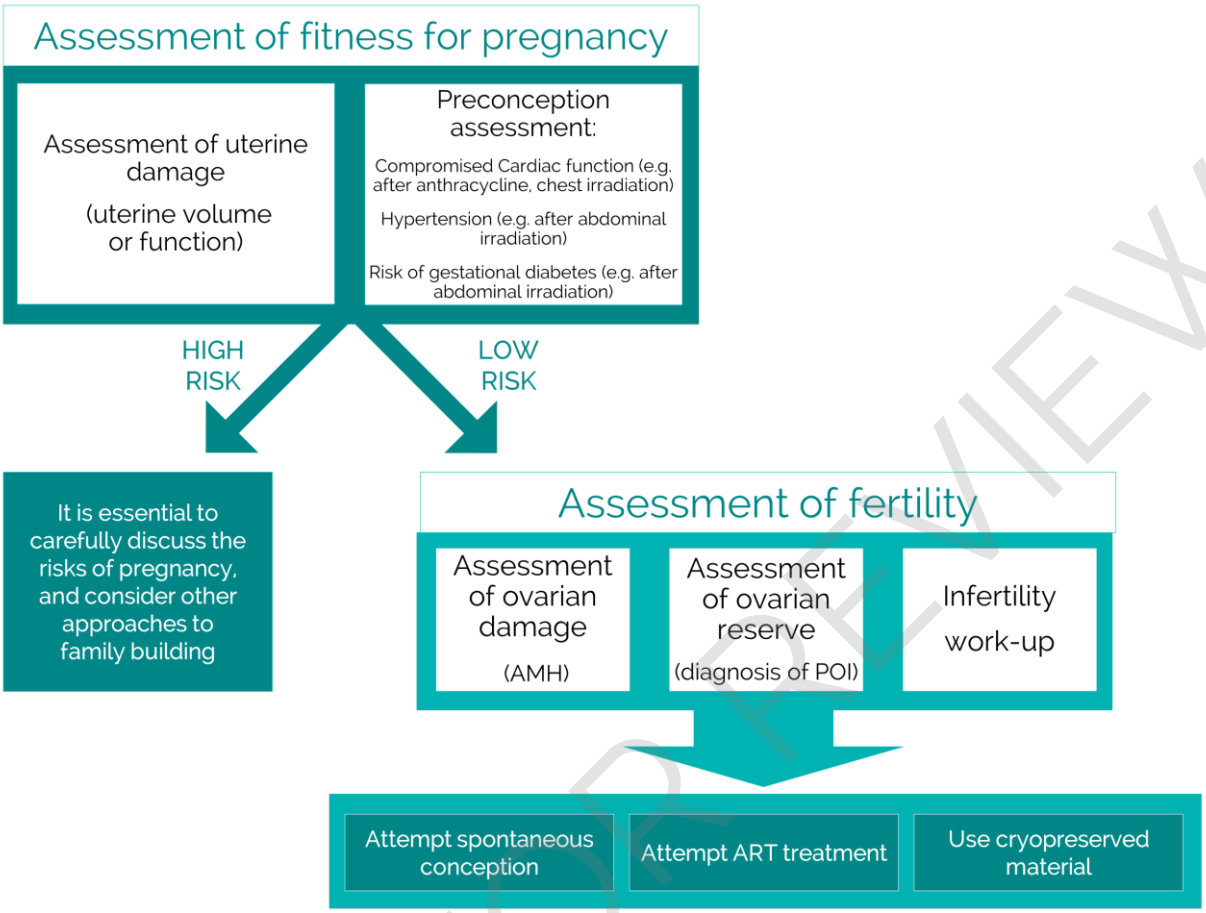
It is important that cancer survivors who present with infertility are fully assessed with consideration of non-cancer related causes of infertility, including their partners. Patients who do not conceive spontaneously or who experience POI and have cryopreserved gametes or ovarian tissue before cancer treatment may be able to conceive via MAR. A risk assessment of their health (including risk of recurrence of disease) through a multidisciplinary discussion on implications for pregnancy is recommended: this should include an oncologist (or other relevant medical specialty) and an obstetrician as well as reproductive medicine specialists. Part of assessing the risk should include an oncological review to assess the safety aspects related to the treatment, with careful review of potential treatment-related effects on cardiovascular and other maternal health (see Figure 5). Frozen embryo replacement in a natural cycle might be recommended instead of in a hormonal replacement treatment cycle for women with oestrogen receptor positive breast cancer, in order to reduce the unnecessary exposure to high levels of oestrogens for a prolonged period of time.

Patients with POI who had not cryopreserved gametes or tissue should be offered support and counselling to deal with infertility and discuss other family building options (see Checklist 4); appropriate guidance can be found in the guidelines for POI (Webber *et al.*, 2016). Psychological counselling, pre-conception antenatal counselling and treatment implication counselling is extremely important and should be offered to all patients. Local guidelines for treatment, taking into account the welfare of the child, should be followed.

How long should patient be in remission?

Although conceiving after a cancer treatment does not increase the risk of cancer recurrence, it is still unknown whether short intervals between treatment and conception might cause poor pregnancy outcomes. Hartnett and colleagues reported outcomes of 4922 births to cancer survivors and concluded that women who conceived ≤1 year after starting chemotherapy had higher risks of preterm birth than control (chemotherapy alone: relative risk [RR] 1.9; 95% CI 1.3-2.7; chemotherapy with radiation: RR 2.4; 95% CI 1.6-3.6); women who conceived ≥1 year after starting chemotherapy without radiation or ≥2 years after chemotherapy with radiation did not have an increased risk overall, although the risk of preterm birth in cervical cancer survivors largely persisted. They concluded that the risk of preterm birth was limited to those survivors who had short intervals between treatment and conception (Hartnett *et al.*, 2018).

Figure 5 Patient re-assessment before attempting pregnancy (with or without the use of stored material) (summary)



Transgender men

Stored material from transgender men can be used in 3 ways, by the patient himself, if he still has a uterus, in a female partner, or in a surrogate (see Checklist 5).

Although there are no papers on re-assessment of transgender men before the use of stored material, medical assessment and the welfare of the child should always be considered, and psychological support offered throughout the pregnancy.

In case of stored material from a transgender man being used by himself, a medical/endocrinological assessment and the type of any ongoing hormonal treatment should be taken into consideration.

Furthermore, the effects of long-term endocrinological treatments, and the start of that treatment (before or after puberty) should be considered, and whether the uterus can sustain a pregnancy.

If using stored material from transgender men by the patient himself is not preferred, alternative family building options (see Checklist 5) depending on context and national regulations should be considered.

Recommendations

Before the use of stored material, fitness for pregnancy should be thoroughly assessed, taking into account treatment late effects, the age of the patient and the interval since treatment.	**STRONG ⊕○○○**

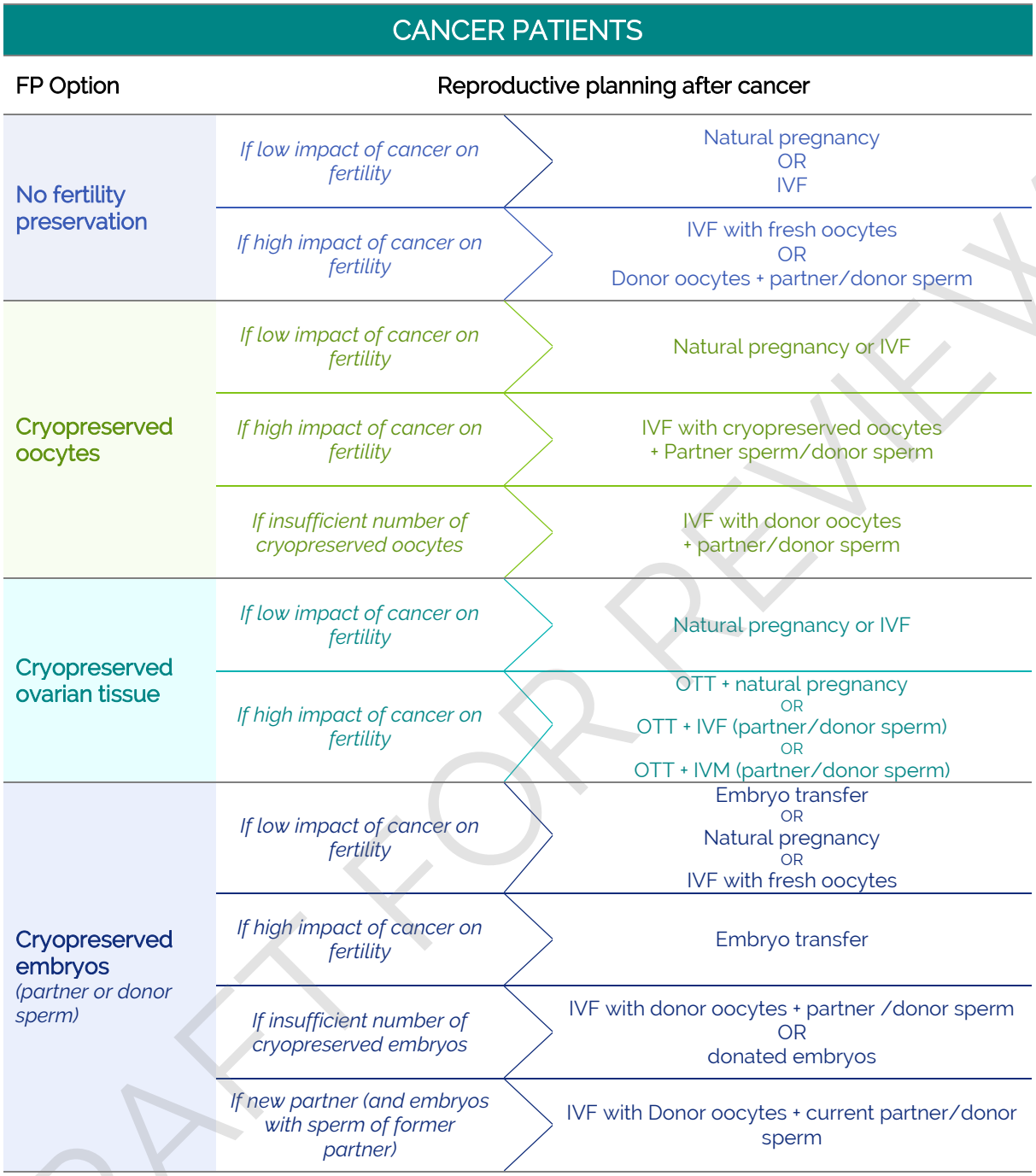
The need for psychological counselling, pre-conception counselling and fertility treatment counselling should be considered for all patients. Local guidelines for counselling should be followed.	**GPP**

Justification

Pregnancy after cancer can be complicated by uterine damage or other late effects of treatments (e.g. chemotherapy, radiotherapy). To predict and prevent possible complications, a thorough assessment of fitness for pregnancy is recommended taking into consideration factors that affect the risk of pregnancy complications (i.e. the type of treatment, the age of the patient and the time since treatment). Additional assessment of ovarian reserve and fertility could be helpful to guide clinical decisions regarding the need to use stored material or the possibility of attempting natural pregnancy.

With the second recommendation, the GDG wants to stress the importance of pre-conception counselling in which the reproductive options are clearly explained. Checklist 4 and Checklist 5 can be helpful to discuss reproductive options after fertility preservation for cancer patients and transgender men, respectively. Adoption is also a possibility for all patients and should be considered where appropriate.

119 **Checklist 4 Reproductive options after fertility preservation for cancer patients**



120 *Abbreviations; FP, fertility preservation; IVF, in vitro fertilization; IVM, in vitro maturation; OTT, Ovarian*
121 *tissue transplantation*

122

123 **Checklist 5 Reproductive options after Fertility preservation for transgender men.**

TRANSGENDER MEN		
FP Option	Reproductive planning with female partner	Reproductive planning with male partner
No fertility preservation	IUI or IVF: Partner oocytes + Donor sperm	IVF: Donor oocytes + Partner sperm + Gestational carrier or TM uterus
Cryopreserved oocytes	IUI or IVF: TM cryopreserved oocytes + Donor sperm	IVF: TM cryopreserved oocytes + Partner sperm + Gestational carrier or TM uterus
Cryopreserved ovarian tissue	OTT/IVM/TM matured oocytes? + Donor sperm	OTT/IVM/TM matured oocytes? + Partner sperm + Gestational carrier or TM uterus
Cryopreserved embryos *(Partner or donor sperm)*	Embryo transfer to partner	Embryo transfer + Gestational carrier or TM uterus

124 *Abbreviations; FP, fertility preservation; IUI, intra-uterine insemination; IVF, in vitro fertilization; IVM, in*
125 *vitro maturation; OTT, Ovarian tissue transplantation; TM, transgender man*

126

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E2. Obstetric outcomes

The focus of this section is to assess whether cancer and its treatment are associated with increased risk of adverse pregnancy outcomes and identify factors that could be used to highlight pregnancies at increased risk.

PICO Question: What is the effect of previous gonadotoxic treatments and underlying conditions on obstetric outcomes?

Reports from large registry data from the Scottish Cancer Registry (van der Kooi *et al.*, 2018), the North Carolina Central Cancer Registry (CCR) (Anderson *et al.*, 2017), the Finnish Cancer Registry (Madanat-Harjuoja *et al.*, 2013, Melin *et al.*, 2019) and the Cancer registry of Norway (Fossa *et al.*, 2005) concluded that women previously treated for cancer had higher rates of postpartum haemorrhage, operative or assisted delivery, and preterm birth (See Tbale 10). Furthermore, their offspring were more likely to require monitoring or care in a neonatal intensive care unit. The risks of early death or stillbirth were not increased after adjustment for prematurity, and there was no increased risk of congenital or chromosomal abnormality (Winther *et al.*, 2012, Nielsen *et al.*, 2018, van der Kooi *et al.*, 2019). Data from the Swedish Cancer Register (10,017 births in female cancer survivors) identified an increased risk of stillbirth within three years after the cancer diagnosis (OR 1.92, 95% CI 1.03–3.57). However, the risk of stillbirth and neonatal death was significantly decreased among second children as compared to the first born, suggesting that any adverse effect associated with cancer treatments may diminish with time (Ji *et al.*, 2016).

A recent meta-analysis of data from cohort studies and registries came to similar conclusions (van der Kooi *et al.*, 2019). Their calculations showed that cancer survivors had an increased risk of prematurity (RR 1.56; 95% CI 1.37-1.77), low birth weight (RR 1.47; 95% CI 1.24-1.73), emergency caesarean section (RR 1.22; 95% CI 1.15-1.30), elective caesarean section (RR 1.38; 95% CI 1.13-1.70), and postpartum haemorrhage (RR 1.18; 95% CI 1.02-1.36). They reported a non-significant difference in small-for-gestational-age-babies (RR 0.99; 95% CI 0.81-1.22), and antepartum haemorrhage (RR 1.06; 95% CI 0.88-1.29). In their meta-analysis, the incidence of congenital abnormalities was significantly higher in children from cancer survivors (RR 1.10; 95% CI 1.02-2.20) (van der Kooi *et al.*, 2019).

Recommendations

Preconception counselling and appropriate obstetric monitoring is recommended in women intending to become pregnant after anticancer treatments.	**STRONG** ⊕⊕⊕◯

Justification

Registry data and cohort study data summarized in a recent meta-analysis show consistently that cancer survivors are at increased risk of postpartum haemorrhage, caesarean section, and preterm birth. The GDG decided that such increased risk justifies preconception counselling and obstetric monitoring. (See also summary Table 11)

Table 10 Overview of data from large registries on obstetric outcomes after cancer

	(van der Kooi *et al.*, 2018)	(Anderson *et al.*, 2017)	(Madanat-Harjuoja *et al.*, 2013)	(Fossa *et al.*, 2005)	(Ji *et al.*, 2016)
	Scotland	North Carolina	Finland	Norway	Sweden
Study group	10,271 nulliparous women diagnosed with cancer before the age of 40 years	21 716 women with a cancer diagnosis between ages 15 and 39 years	25 784 males and females	8644 women after diagnosis in cancer patients aged 15 to 45	1,977 cancer survivors who had given birth before / after their cancer diagnosis
Control group	General population	General population	44 611 full and half siblings of these patients		General population (without cancer)
BIRTH					
Antepartum haemorrhage	No difference (RR 1.13; 95% CI 0.86–1.50)				
Postpartum haemorrhage	**Increased** (RR 1.42; 95% CI 1.29–1.55)				
Operative or assisted delivery – elective	**Increased** (RR 1.59; 95% CI 1.35–1.88)	**Increased** (PR 1.08; 1.01-1.14)		**Increased** (OR 2.3; 95% CI 1.9-2.7)	
Operative or assisted delivery – emergency	**Increased** (RR 1.20; 95% CI 1.08–1.34)				
PERINATAL OUTCOMES					
Small for gestational age	**Decreased** (RR 0.82; 95% CI 0.68–0.98)	No difference (PR 0.97; 0.85-1.11)			
Low Apgar score (<7)		No difference (PR 1.18; 0.87-1.61)			
Low birth weight	No difference (RR 1.15; 95% CI 0.94–1.39)	Increased (PR 1.59; 95%CI 1.38-1.83)		**Increased** (singletons) (OR 2.5; 95% CI 2.0-3.2)	
Preterm birth	**Increased** (RR 1.32; 95% CI 1.10–1.59)	**Increased** (PR 1.52; 95% CI 1.34-1.71)		**Increased** (singletons) (OR 2.8; 95% CI 2.3-3.4)	
Early preterm birth		**Increased** (PR 2.03;95%CI 1.62-2.55)			
Need for intensive care or neonatal monitoring	**Increased** (RR 1.03; 95% CI 0.90–1.19)		**Increased** (OR 1.90; 95% CI 1.65 – 2.19)		
Perinatal death (< 7 days after live birth)			No difference (OR 1.35; 95% CI 0.58 – 3.18)	No difference (OR 1.2; 95% CI 0.6-2.4)	
Neonatal death (< 28 days after live birth)			No difference (OR 1.40; 95% CI 0.46 – 4.24)		No difference (OR 1.13; 95% CI 0.80–1.60)
Early death (< 1 year after birth)			No difference (OR 1.11; 95% CI 0.64 – 1.93)		
Stillbirth			No difference (OR 1.15; 95% CI 0.61 – 2.19)		No difference (OR 1.27; 95% CI 0.95–1.68)
Congenital abnormalities	No difference (RR 1.01; 95% CI 0.85–1.20)			No difference (OR 0.6; 95% CI 0.4-1.0)	

PR = prevalence ratio; RR = relative risk; OR = odds ratio

Effect of chemotherapy

No systematic reviews were found on the effect of different chemotherapy regimens in adult women on subsequent pregnancy. Recent analysis suggests that chemotherapy is not associated with adverse pregnancy outcomes (van Dorp *et al.*, 2018).

Akhtar and colleagues retrospectively assessed 176 patients (age 14-40 years) who underwent high dose chemotherapy and autologous stem cell transplant without total body irradiation (TBI) for diffuse large B-cell lymphoma and Hodgkin lymphoma (Akhtar *et al.*, 2015). Twenty-six patients (65%) became pregnant 50 times (range 1-6 times), resulting in 43 (86%) live births, 7 (14%) miscarriages, and 1 still birth (at 28 weeks). There was a significantly higher incidence of successful pregnancies after autologous stem cell transplant in patients younger than 40 years. Other single studies were of very small patient groups, precluding accurate interpretation.

Large prospective cohort and population-based studies have evaluated the effects of chemotherapy for childhood cancer on subsequent pregnancy outcomes, whereas data are more limited for adult cancer patients. One recent publication reported outcomes of 4922 births to cancer survivors and concluded that women who conceived ≥1 year after starting chemotherapy without radiation or ≥2 years after chemotherapy with radiation did not have an increased risk of preterm birth (Hartnett *et al.*, 2018). Women who conceived ≤1 year after starting chemotherapy had higher risks of preterm birth than controls (chemotherapy alone: RR 1.9; 95% CI 1.3-2.7; chemotherapy with radiation: RR 2.4; 95% CI 1.6-3.6).

Recommendation

An interval of at least 1 year following chemotherapy completion should be considered before attempting a pregnancy in order to reduce the risk of pregnancy complications	**STRONG** ⊕○○○

Justification

In general, there was an increased risk of preterm birth in women after cancer treatment (see previous section). The study of Hartnett, looking at the impact of chemotherapy, shows that this effect may be linked to the time interval between the end of chemotherapy and the pregnancy. Such information should be included in preconception counselling.

Effect of Pelvic radiotherapy

There are robust data from that radiotherapy to a field that includes the uterus is associated with adverse pregnancy outcomes in women who had been exposed during childhood and adolescence, but the data following adult exposure are much more limited. Females treated with pelvic radiation for childhood cancers have an increased rate of uterine dysfunction leading to pregnancy loss, preterm birth and low birth weight (Critchley and Wallace, 2005). These pregnancy-related complications are related with reduced uterine volume, damage of uterine vessels, myometrial fibrosis, endometrial injury (Critchley and Wallace, 2005) (Teh *et al.*, 2014). Doses of 14 to 30Gy can lead to irreversible uterine function in young female patients (Critchley and Wallace, 2005).

A large retrospective cohort study, performed between 1970 and 1986, enrolled 1774 women younger than 21 years at initial cancer diagnosis, who had survived for at least 5 years after diagnosis and who had received radiotherapy, found that high-dose pelvic irradiation can permanently impair growth and blood flow to the uterus resulting in a reduced uterine volume; these effects of radiation are dependent on age (Signorello *et al.*, 2010). Sixty stillbirths or neonatal deaths, and 3077 live births were reported. Uterine or ovarian irradiation with doses ≥2.5 Gy greatly increased the risk of stillbirth or neonatal death (12-fold) in women treated before menarche. Therefore, careful management is warranted for pregnant women treated with high doses of pelvic irradiation, particularly before they have reached puberty

In a study reporting on the effect of adulthood radiation effect on pregnancy, the incidence of spontaneous abortion (37% versus 7%) and preterm birth (63% versus 18%) were significantly higher in TBI recipients when compared to the chemotherapy-only group (Sanders *et al.*, 1996). The 13 preterm births resulted in 10 low birth weight (1.8 to 2.24kg) and three very low birth weight (≤ 1.36kg) infants, for an overall incidence of 25%, which is higher than the expected incidence of 6.5% for the general population. Four Gy appears to be the threshold dose.

Radiotherapy-induced structural and functional changes to the uterus (> 5Gy) may adversely affect implantation and maintenance of pregnancy increasing the risk of placental attachment disorders (placenta accreta or placenta percreta), low birth weight (OR 3.64; 95% CI 1.33-9.96; in survivors after abdominopelvic radiation; OR 6.8; 95% CI 2.1-22.2); small for gestational age (OR 4.0; 95% CI 1.6-9.8) ; preterm birth (OR 3.5; 95% CI 1.5-8.0); and perinatal death and foetal malposition (Tarin *et al.*, 2016).

In conclusion, uterine exposure to radiotherapy during childhood reduces adult uterine volume and leads to an increased risk of pregnancy complications and adverse pregnancy outcomes. Preconceptional assessment and appropriate obstetric monitoring is warranted (van de Loo *et al.*, 2019).

Recommendation

Radiotherapy to a field that included the uterus increases the risk of pregnancy complications; this risk is age and dose dependent. These pregnancies should be treated as high risk and managed in a centre with advanced maternity services.	**STRONG** ⊕○○○

Justification

Most of the reports on pregnancy outcomes after pelvic radiotherapy are based on patients receiving treatment for childhood cancer. Although these reports provide only indirect evidence, a negative impact of pelvic radiotherapy in adulthood can be expected. As such, pregnancies in patients who received previous pelvic radiotherapy could be associated with severe complications. The GDG decided to strongly recommend careful follow-up of these pregnancies, irrespective of whether the radiotherapy was received in childhood or when adult.

Research recommendation:

The effect of pelvic radiotherapy in adults on pregnancy outcomes should be further investigated.

Breast cancer

A systematic review and meta-analysis reported on associations between maternal breast cancer and adverse delivery outcomes. The reviewers concluded that maternal breast cancer was associated with an increased risk of preterm birth (pooled RR 1.82; 95% CI 1.44-2.30) based on 7 studies (n=6,687,579 patients and controls) and low birth weight (pooled RR 1.41; 95% CI 1.15-1.74) based on 5 studies (n=6,687,103 patients and controls) (Sun *et al.*, 2018). However, when the analysis for preterm birth was stratified by publication year, the risk associated with breast cancer appeared larger among studies published before 2010 (RR 2.18; 95% CI 1.83–2.60) compared with studies published after 2010 (RR 1.42; 95% CI 1.04–1.94). After excluding each study individually, the sensitivity analysis confirmed the significant associations between history of breast cancer and increased risk of preterm birth and low delivery weight, suggesting high stability in the meta-analysis results. A recent large registry study including 18,280 women with history of breast cancer from South Korea found that breast cancer survivors had a lower probability of full-term delivery (adjusted OR 0.78; 95% CI 0.68-0.90) and a higher frequency of preterm birth (adjusted OR 1.33; 95% CI 1.06-1.65) than controls (Lee *et al.*, 2019).

A systematic review and meta-analysis conducted by Hartman and colleagues included 19 studies assessing the overall and disease-free survival of women where pregnancy occurred after breast cancer diagnosis. These women (n=1829) had a significantly reduced risk of death compared to the

controls (n=21,907) who did not conceive (HR 0.63; 95% CI 0.51-0.79) (Hartman and Eslick, 2016). The reviewers recalculated the ratios taking into consideration the "healthy mother effect"[5] with the same conclusion; women who became pregnant after a diagnosis of breast cancer had a reduced risk of death (HR 0.65; 95% CI 0.52–0.81). Moreover, there was a decreased risk of recurrence and disease progression for these women (HR 0.93; 95% CI 0.68–1.28). After the publication of this meta-analysis, 3 studies were published. Lambertini and colleagues assessed the prognostic value of pregnancy after breast cancer overall and according to hormone receptor status (Lambertini *et al.*, 2018). At a median follow-up of 7.2 years after pregnancy (approximately 10 years after breast cancer diagnosis), no difference was observed in disease-free survival between patients with or without a pregnancy after estrogen receptor (ER)-positive (HR 0.94; 95% CI 0.70-1.26) or ER-negative (HR 0.75, 95% CI 0.53-1.06) breast cancer. There was no difference in overall survival in patients with ER-positive disease (HR 0.84, 95% CI 0.60-1.18), while women with ER-negative breast cancer with a subsequent pregnancy showed better overall survival (HR 0.57; 95% CI 0.36-0.90). Iqbal and colleagues performed a retrospective study (n=7553) looking at the association between timing of pregnancy with survival after breast cancer. Pregnancy did not adversely affect the 5-year survival rate in women with breast cancer (age-adjusted HR 0.22; 95% CI 0.10-0.49) and adjusting for ER status did not influence the results (Iqbal *et al.*, 2017). A retrospective analysis conducted within the large adjuvant ALTTO[6] randomized trial reported on the prognostic effect of having a pregnancy after HER2-positive early breast cancer. With an extended Cox model with time-varying covariates to account for a guarantee-time bias (to account for a possible 'healthy mother' effect), the study did not show any significant difference in disease-free survival (adjusted HR 1.12; 95% CI 0.52-2.42) between young patients with a pregnancy (n=85) and those without (n=1,307) (Lambertini *et al.*, 2019).

Adjuvant treatment and pregnancy

A recent review summarized data from 238 cases of tamoxifen use during pregnancy. Abnormal foetal development was reported in 21 of 167 pregnancies (12.6%) with known outcome (Schuurman *et al.*, 2019). The overall miscarriage rate was 6.7%. The safety and feasibility of temporary interrupting anti-estrogen therapy (for up to 2 years) for allowing pregnancy attempts, with subsequent resumption of therapy is currently being investigated in the POSITIVE trial. Results are expected in 2028 (https://clinicaltrials.gov/ct2/show/study/NCT02308085).

Recommendations

After completion of the recommended treatment, pregnancy is safe in women who have survived breast cancer. This is independent of estrogen receptor status of the tumour.	**STRONG** ⊕⊕○○
Pregnancy after treatment for breast cancer should be closely monitored, as there is an increased risk of preterm birth and low birth weight. Patients should be informed about these risks.	**STRONG** ⊕⊕⊕○
Reliable non-hormonal contraception is mandatory during tamoxifen treatment. It is recommended to stop tamoxifen for at least 3 months before attempting pregnancy.	**GPP**

[5] The "healthy mother effect" is a selection bias where only women who have had favorable outcomes following diagnosis are likely to conceive

[6] the Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization trial (ALTTO)

Justification

Data from a meta-analysis of 19 studies and 3 more recent reports consistently show that there is no negative effect of pregnancy on disease-free survival or overall survival in women after a previous diagnosis of breast cancer. Analysis of subgroups of patients with different breast cancer subtypes (based on ER status or HER2 positivity) did not find any detrimental survival effect for a post-treatment pregnancy.

Although considered safe for the mother, there seems to be an association between maternal breast cancer and increased risk of preterm birth and low delivery weight (data from a meta-analysis). The GDG stresses that patients should be informed and monitored more closely if pregnant.

The safety impact of interrupting tamoxifen for having a pregnancy is the topic of an ongoing trial. So far, evidence from a limited number of cases has shown that tamoxifen during pregnancy can increase the risk of foetal abnormalities. In the absence of reliable data, women are generally advised to stop tamoxifen treatment and wait for minimum 3 months before attempting conception to allow appropriate wash out period from the drug.

Gynaecological cancers

Endometrial cancer

Two systematic reviews looked at pregnancy outcomes after endometrial cancer. Gunderson and colleagues summarized data from 38 studies reporting on 315 women after hormonal treatment for grade 1 adenocarcinoma or endometrial hyperplasia of which 114 conceived at least once and 117 live births occurred. Reproductive outcomes (i.e. live births) did not differ between the cohorts with different endocrine treatments (Gunderson *et al.*, 2012). This was subsequently confirmed in a study of pregnancy outcomes after fertility-sparing management using oral progestin for young women with endometrial cancer (Park *et al.*, 2013). In 51 pregnancies in 70 women, they reported a miscarriage rate of 24%, an ectopic pregnancy rate of 2.8% and a preterm birth rate of 11.5% (Park *et al.*, 2013).

The second review, overlapping partly in terms of included studies, analysed 50 patients with early stage endometrial cancer (grade 1 and 2) who conceived after conservative treatment (progestogen treatments). There was a significant increase in hypertensive disorders, preterm birth, multiple pregnancies and caesarean section in women who conceived after ART (n=14) compared to women who conceived spontaneously or had ovulation induction with intrauterine insemination (n=36) (Chao *et al.*, 2011).

Oncologic outcomes were also discussed in the review by Gunderson (45 studies, 391 patients) (Gunderson *et al.*, 2012). The reviewers reported a recurrence rate of 35.4% in the carcinoma cohort and 23.2% in the hyperplasia group, with a median time to recurrence of 24 months (range from 4 to 72 months) (Gunderson *et al.*, 2012). The reviewers did not investigate a possible association between recurrence and pregnancy.

The report of the ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer suggests women should aim to conceive soon after documented tumour regression (Colombo *et al.*, 2016). In patients where this is not possible, continuation of hormonal suppression is advocated until conception can be attempted. After completion of childbearing, it is suggested to apply standard treatment for endometrial cancer, i.e. hysterectomy.

Ovarian cancer

Smaldone and colleagues performed a retrospective analysis of reproductive-age women (18-45 years old) with stage IA to stage IIC ovarian neoplasms (n = 161): thirteen women successfully conceived 23 pregnancies, with 18 documented live births (Smaldone *et al.*, 2010).

Park and colleagues retrospectively analysed women with borderline ovarian tumour who underwent fertility-sparing surgery versus radical surgery. Of the patients undergoing fertility-sparing surgery, 27 out of 184 patients conceived and had 32 singleton and 1 twin delivery, all

healthy. The rate of recurrence in this series it was 5.1% in the fertility sparing versus 4.9% in the radical surgery group, respectively (Park *et al.*, 2009). Janda and colleagues summarized the data from this study and 3 others (in a narrative review). They reported that out of 158 women who attempted to conceive after fertility-sparing surgery for ovarian cancer, 121 attained pregnancy (76.5%). There were 148 live births.

Another systematic review reported on recurrence based on 39 studies with 1150 patients after fertility-sparing surgery for ovarian cancer. Recurrence was reported for 139 patients, with an overall recurrence rate of 11% (Bentivegna *et al.*, 2016a). This has been recently supported by a retrospective analysis reporting on that 56 babies born to 40 malignant ovarian germ cell tumour survivors after fertility sparing treatment (Tamauchi *et al.*, 2018).

Cervical cancer

Traditionally, early stage cervical cancer in young women who want to maintain their pregnancy, is treated by radical trachelectomy, i.e. vaginal or abdominal removal of the cervix with part of the vagina and parametrium. Three systematic reviews refer an overall live birth rate of 68-70%. However, these pregnancies are complicated by pregnancy loss (14.8%) and preterm birth (26.6%), including extreme preterm birth (less than 28 to 30 weeks) (Bentivegna *et al.*, 2016b, Kyrgiou *et al.*, 2017, Zhang *et al.*, 2017). Transabdominal cerclage (TAC) of the uterine cervix has been proposed in order to reduce the risks of preterm birth, but a retrospective review of 11 cases in which TAC was performed reported risks of complications as a result of the use of non-absorbable thread and the need for two extra laparotomies (Ishioka *et al.*, 2018). A Danish study (included in the reviews) reported that 25% of patients required ART (Hauerberg *et al.*, 2015).

An alternative approach, currently being prospectively evaluated, is the neoadjuvant administration of chemotherapy, allowing tumour reduction and less radical surgery (including conisation or cervical amputation) and possibly resulting in better obstetric outcomes (Plante *et al.*, 2019).

Recommendations

Women with endometrial cancer, should be followed up for high-risk pregnancy and monitored by an oncologist due to the risk of relapse.	**STRONG** ⊕○○○
The risk of preterm birth is increased after treatment for early cervical cancer and these pregnancies should be treated as high risk and managed in a centre with advanced maternity services.	**STRONG** ⊕⊕○○

Justification

Evidence on pregnancy outcomes after fertility-sparing treatment of endometrial cancer suggest that there is an increased risk of obstetric complications, which supports a recommendation for careful follow-up of these pregnancies. The standard treatment (i.e. hysterectomy) is postponed in patients with endometrial cancer, until they have completed their child wish. With the significant risk of recurrence in patients with endometrial cancer after fertility-sparing treatment only (irrespective of pregnancy), additional follow-up by an oncologist is recommended. The recommendation for increased monitoring is considered proportionate to the risk, feasible and acceptable. More information on the timing of pregnancy was provided in the ESMO-ESGO-ESTRO guidelines (Colombo et al., 2016)

From 3 systematic reviews, there seems to be a significant risk of preterm birth rate after treatment for cervical cancer. Preterm birth rates of 26.6% were reported. For safety reasons, precautions should be taken.

253 Table 11 Overview of specific guidance per type of cancer (Summary)

Disease	Treatment	Obstetric risks	Recommendations for care before pregnancy	Recommendations for care during/after pregnancy
All cancers	(independent of treatment)	Cancer survivors are at increased risk of postpartum haemorrhage, caesarean section, and preterm birth.	Preconception counselling	Appropriate obstetric monitoring
	Chemotherapy started <1year before conception	Increased risk of preterm birth	Patients should be advised about these risks	
	Pelvic radiotherapy (field includes the uterus)	Increased risk of (possibly severe) pregnancy complications		Treat pregnancy as high risk in a centre with advanced maternity services
Breast cancer	(independent of treatment)	Increased risk of preterm birth and low birth weight	Pregnancy is safe	
	If chemotherapy started <1year before conception	Increased risk of preterm birth	Patients should be advised about these risks	
	With adjuvant therapies	Risks unclear	Stop tamoxifen for at least 3 months before attempting pregnancy	
Endometrial cancer	Fertility-sparing surgery	Increased risk of obstetric complications + possible recurrence awaiting definitive treatment (Hx)	Inform patients that better outcomes are seen when conception occurs soon after documented tumour regression.	High-risk pregnancy, patients are to be monitored by an oncologist, due to the risk of relapse
	Pelvic radiotherapy (field includes the uterus)	Risk of (possibly severe) pregnancy complications		Treat pregnancy as high risk in a centre with advanced maternity services
Ovarian cancer	Fertility-sparing surgery	No evidence		Follow general advice for cancer survivors
Cervical cancer	Radical trachelectomy	Risk of pregnancy loss and preterm birth		Treat pregnancy as high risk in a centre with advanced maternity services
	Pelvic radiotherapy (field includes the uterus)	Risk of (possibly severe) pregnancy complications		Treat pregnancy as high risk in a centre with advanced maternity services

254 *Abbreviations; Hx, hysterectomy*

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Other cancers

Haggar and colleagues retrospectively analysed 232 first pregnancies in survivors of colorectal cancer who underwent surgery and compared them with randomly selected pregnancy (without a history of maternal cancer) (Haggar *et al.*, 2013). Previous colorectal cancer, particularly rectal and radiation-treated tumours, appears to confer an increased likelihood of adverse outcomes (postpartum haemorrhage, caesarean delivery, low APGAR score, and need for special neonatal care) in pregnancy. In women that had open cancer surgery, there was an elevated risk of gastrointestinal obstruction during pregnancy (OR 1.17; 95% CI 1.08-1.27), pregnancy loss (OR 1.26; 95% CI 1.04-1.52), and prolonged postpartum hospitalization (OR 3.11; 95% CI 1.42-7.73) compared to the control group. Laparoscopic surgery had less impact on these adverse gestational outcomes. Women undergoing rectal surgery also had an increased risk of adverse outcomes compared with those who underwent colonic resection.

Analysis of other individual cancer types (thyroid cancer: (do Rosario *et al.*, 2006) and osteosarcoma: (Longhi *et al.*, 2000)) are based on very small numbers of patients, precluding accurate analysis.

A recent prospective cohort study comparing women who conceived after oocytes donation with or without history of cancer found that the risks of preterm birth and pre-eclampsia in women with prior cancers significantly exceed those of women without cancer history undergoing similar treatments (Marklund *et al.*, 2018).

Recommendation

Women previously treated for cancer require individual assessment of their obstetric risks and potential additional obstetric surveillance.	**STRONG** ⊕○○○

Justification

Evidence on obstetric outcomes in women previously treated for cancers other than breast cancer or gynaecological malignancy are all observational and reported in small studies. Large registry data, although not specific for a certain type of malignancy, have shown increased maternal and neonatal risks associated with these pregnancies and support a cautious approach of individual assessment and obstetric surveillance in women previously treated for cancer.

Transgender men

While the cryopreservation of gametes is rapidly growing in transgender patients, there is very limited information on pregnancy in transgender men. Obedin-Maliver and colleagues identified and reviewed 3 studies and highlighted psychological issues experienced by transgender men contemplating pregnancy or bearing a child (Obedin-Maliver and Makadon, 2016). Twenty-five (61%) reported testosterone use prior to pregnancy. but pregnancy, delivery, and birth outcomes did not differ according to prior testosterone use. Parents reported both internal and external struggles. Internal challenges were typified by the conflict between one's identity as male and or gender variant and "social norms that define a pregnant person as woman and a gestational parent as mother." Regarding the external world, contemplation and experience of pregnancy involved a constant tension about needing to "manage others' perceptions and either disclosing or not disclosing what they were experiencing."

Pregnancy was reported to improve gender dysphoria in some cases whereas in others there was an increase in dysphoria, which could continue into the postpartum period (Light *et al.*, 2014). Participants repeatedly expressed a desire for more information regarding fertility options and access to reproductive health care providers who respect, support, and understand their gender identity.

According to a recent review, the psychological impact of pregnancy on gender dysphoria is unknown (Brandt *et al.*, 2019). The profoundly gendered experience of pregnancy, including labor and delivery, is likely to exacerbate the dysphoria, but the prevalence and long-term impact of depression during pregnancy and postpartum in transgender men is unknown.

Transgender men can become pregnant both intentionally and unintentionally. Hence, healthcare providers need to be equipped with counselling on reproductive needs from preconception (including careful discussion of contraceptive needs) to the postpartum period. Additional support and guidance from mental health colleagues may be beneficial. In addition, the obstetrician needs to ensure a seamless transition from postpartum care to the team of gender affirming providers that manage his medical and gynaecologic healthcare needs (Brandt *et al.*, 2019).

Recommendation

Healthcare professionals should have a high level of awareness of the risk of depression and increased dysphoria during and after pregnancy care for transgender men	**WEAK** ⊕○○○

Justification

Based on some evidence for a high prevalence of depression in transgender people, and combined with possible additional stress from pregnancy, increased rates of postnatal depression can be expected in transgender men. The GDG recommends healthcare professionals are aware of this.

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PART F: Ongoing developments in FP

To increase the spectrum of fertility preservation (FP) options, innovative technologies and novel in vitro avenues are continually being developed. Some of those may lead not only to more effective FP strategies, but also to a broader range of treatments for infertility.

The goal of this narrative is to provide an overview of challenging concepts and emerging technologies that may be translated to alternative FP strategies in the future.

NARRATIVE QUESTION: WHAT ARE ONGOING DEVELOPMENTS WITH REGARDS TO FERTILITY PRESERVATION?

Technologies involving transplantation into the patient

Technologies involving transplantation of ovarian tissue or cells as well as non-ovarian cells aiming to restore ovarian function (both reproductive and when possible endocrine) in the patient may prove applicable in the broader context of infertility.

Transplantation of the whole ovary after cryopreservation

Removing, cryopreserving and transplanting the whole ovary would seem the preferred strategy to restore functionality (Gosden, 2008). However, there are several obstacles that need to be overcome: it remains a challenge to cryopreserve the whole ovary without inducing cryoinjury, revascularization of the transplanted ovary remains difficult due its complex dynamic architecture and the reintroduction of malignant cells in the patient cannot be excluded. To date, there are no reports of live births resulting from the transplantation of frozen-thawed ovine (Onions *et al.*, 2013) or human whole ovaries (Ladanyi *et al.*, 2017). Hence, this procedure remains to be optimized (Ali Mohamed, 2017).

Optimizing the use of transplanted ovarian cortex tissue

In vitro activation

In vitro activation (IVA) is an experimental procedure that has been offered as infertility treatment to women with premature ovarian insufficiency (POI), whose ovaries still contain a pool of (dormant) primordial follicles. This procedure has resulted in several reported live births (4 live births from 51 patients) (Kawamura *et al.*, 2013, Suzuki *et al.*, 2015, Zhai *et al.*, 2016, Fabregues *et al.*, 2018). IVA involves the mechanical fragmentation of the ovarian cortex tissue followed by culture to stimulate the protein kinase B (PKB) signalling pathway. The cultured IVA-fragments are placed in a pouch beneath the serosa of the fallopian tubes and the growing oocytes need to be aspirated to be used in medically assisted reproduction (MAR). IVA has not been reported in the context of FP (Kawamura *et al.*, 2015, Cordeiro *et al.*, 2016, Fabregues *et al.*, 2018).

Reducing ischemia by promoting revascularization

After ovarian tissue transplantation (OTT), the process of revascularization (by vasculogenesis and angiogenesis) takes about 10 days, during which the ovarian cortex tissue undergoes a high rate of follicular loss due to ischemia (Baird *et al.*, 1999, Van Eyck *et al.*, 2010). Accelerated revascularization of the ovarian cortex tissue, by embedding in biomaterial scaffolds of decellularized (extracellular) tissue matrix (Oktay *et al.*, 2016) or hydrogel matrix (Chiti *et al.*, 2018b) may decrease the effects of ischemia after transplantation. This coating in a biomaterial scaffold can also serve as a vehicle to include i) angiogenic factors or molecules, such as VEGF, FGF2, HBP, SIP (Shikanov *et al.*, 2011, Soleimani *et al.*, 2011, Friedman *et al.*, 2012, Kang *et al.*, 2016) ii) patient's endothelial or stromal cells, isolated from either the ovarian cortex or medulla (Dath *et al.*, 2011, Stimpfel *et al.*, 2014, Soares *et*

al., 2015, Man et al., 2018); iii) patient's mesenchymal stromal cells isolated from non-ovarian tissue, such as bone marrow or adipose tissue (Manavella et al., 2018, Shojafar et al., 2018); or iv) cells isolated at the time of birth, from umbilical cord blood or amniotic fluid (reviewed in (Fazeli et al., 2018, Sheikhansari et al., 2018)), as additional factors to induce faster revascularization of the graft.

Xenotransplantation of human mesenchymal stromal cells (MSCs) derived from bone marrow, together with human ovarian cortex tissue supported by a 3D-scaffold into immune compromised mice resulted in higher survival of primordial follicles and enhanced revascularization (Xia et al., 2015, Zhang et al., 2017). However, the follicles remained primordial, suggesting limited effect.

Eliminating residual malignant cells

Technologies being developed to eliminate residual malignant cells that may be present in the ovarian cortex tissue include purging or treatment for 24 hours in vitro with Verteporfin to eliminate artificially-introduced malignant cells in ovarian cortex tissue (Mulder et al., 2019). Removal of the leukemic cells can be achieved by dissociating the ovarian cortex tissue and conducting a series of washing steps on the follicle suspension that is afterwards embedded in a hydrogel matrix (fibrin) into the mouse xenograft model (Soares et al., 2015, Soares et al., 2017). While elimination of malignant cells was effective (Soares et al., 2017), key performance indicators such as follicle development and oocyte quality were not reported.

Transplantation of follicles isolated from ovarian cortex tissue as bioprosthetic ovaries

To restore both endocrine activity and fertility, technology is being developed to generate a transplantable artificial or bioprosthetic ovary. The 3D-scaffolds provide physical support to allow the dynamic cellular interactions within the follicles during follicular growth as well as revascularization and remodelling (Amorim and Shikanov, 2016, Vanacker and Amorim, 2017, Chiti et al., 2018a).

In mice, a 3D-scaffold of fibrin or a combination of fibrin/collagen and fibrin/alginate, with VEGF treatment transplanted into the ovarian bursa of surgically sterilized mice, was shown to support the maturation of follicles from ovaries from 6-day-old mice, which after natural mating gave rise to viable offspring (Kniazeva et al., 2015). 3D-printing with gelatine-ink was used to produce a microporous scaffold (2mm in size) that was seeded with isolated follicles from 16-day-old mice. After transplantation, these bioprosthetic ovaries were vascularized and restored ovarian function successfully resulting in pups born through natural mating as well as normal lactating behaviour suggestive of adequate endocrine function by the corpus luteum (Laronda et al., 2017).

Human preantral follicles embedded in a fibrin-based 3D-scaffold were xeno-transplanted into adult mice for 7 days and although high follicular loss was reported, the retrieved (preantral) follicles seemed viable and showed proliferating granulosa cells (Paulini et al., 2016, Chiti et al., 2017). Decellularized ovarian tissue can also be used as a scaffold; decellularized human ovaries seeded with ovarian cells from adult rat then xenografted for 4 weeks, supported vascularization of the graft and increased endocrine function, but only primordial and primary follicles were reported (Hassanpour et al., 2018).

Transplantation of isolated cells into the remaining (gonadotoxic-exposed) ovary

Patient (autologous) cells isolated from non-ovarian tissues

Mesenchymal stromal cells (MSCs), also referred to as mesenchymal stem cells, may regulate vascularization and immune response, contributing to organ homeostasis, tissue remodelling and wound repair (Sobhani et al., 2017, Fazeli et al., 2018, He et al., 2018, Yoon, 2019) and can be directly isolated from bone marrow, adipose and many other tissues (Fazeli et al., 2018, Sheikhansari et al., 2018, Yin et al., 2018, Yoon, 2019).

Injection (intravenous or intraovarian) of bone marrow from adult mice into gonadotoxic-exposed adult mice improved pregnancy rates due to the recruitment of existing (dormant) follicles (Santiquet *et al.*, 2012), and similar results have been reported with human MSCs from umbilical cord blood and bone marrow injected into gonadotoxic-exposed adult rodents (Mohamed *et al.*, 2018, Zheng *et al.*, 2019). In general, the injection of human MSCs from different origins into rodent models has demonstrated promising results regarding increased ovarian systemic function (fertility and fecundity), leading to activation of existing dormant oocytes in the gonadotoxic-exposed animal host (Fazeli *et al.*, 2018, Yoon, 2019). Injection of MSC conditioned medium (containing exosomes) alone may be sufficient to improve ovarian function (Huang *et al.*, 2018).

Studies have reported the intraovarian injection of human MSCs from bone marrow in women with idiopathic POI (Edessy *et al.*, 2016, Gabr *et al.*, 2016), but the absence of control groups precludes reliable interpretation.

Patient (autologous) cells isolated from the ovarian cortex tissue

There is a robust body of evidence that mouse adult gonadotoxic-exposed ovaries can support *de novo* folliculogenesis, provided suitable cells are transplanted (Zhang *et al.*, 2011, Zhang *et al.*, 2012, Wu *et al.*, 2017). Transplanted mouse foetal ovarian cells (most probably germ cells) have the ability to generate MII oocytes in foetal-derived follicles in mouse adult (gonadotoxic-exposed) ovaries (Zhang *et al.*, 2012). However, although restored ovarian function as well as the birth of pups have been reported, it remains unclear whether transplanted mouse postnatal (neonatal and adult) ovarian cells - that are not oocytes or germ cells - have the ability to differentiate into oocytes that can mature to functional MII oocytes after transplantation into mouse adult (gonadotoxic-exposed) ovaries (Zhang *et al.*, 2011, Zhang *et al.*, 2012, Xiong *et al.*, 2015, Wu *et al.*, 2017).

The potential of cells isolated from human ovarian cortex to generate oocytes has been investigated (White *et al.*, 2012). However, efforts to evaluate the maturation potential of these cells have not been reported.

Patient (autologous) cells reprogrammed to induced pluripotent cells (iPSCs)

Human patient-specific induced pluripotent stem cells (iPSCs) can be obtained from patient (somatic) cells after a period of several weeks of reprogramming in the laboratory.

Mouse iPSCs have been differentiated in vitro to germ cells precursors and after a 2-day period of in vitro coculture, aggregation with foetal female gonads can initiate meiotic entry. Those aggregates were transplanted under the ovarian bursa and after 4-weeks GV stage oocytes were recovered, matured, and fertilized in vitro giving rise to live offspring (Hayashi *et al.*, 2012, Hayashi and Saitou, 2013). Application of this protocol to human iPSCs, including the co-culture of human iPSCs with mouse or human foetal gonads, followed by xenotransplantation to mouse models to evaluate oocyte production have not been reported.

The use of human iPSCs for clinical applications has major challenges including the generation of iPSC under current good manufacturing practice conditions, obtaining sufficient cells to apply to humans, the time necessary to generate patient-specific iPSCs and associated expenses, currently suggesting limited feasibility for clinical applications (Eguizabal *et al.*, 2019).

Technologies that do not involve transplantation

Technologies that do not involve transplantation into the patient have broader applications but are currently less developed. The generation of viable embryos from in vitro-cultured ovarian cortex tissue, containing primordial or primary follicles, has been demonstrated so far in mice (Guzel and Oktem, 2017, Herta *et al.*, 2018) and macaque (Xu *et al.*, 2018b). Recent advances in our knowledge of the molecular signature of human oocytes at different stages of maturation using single-cell omics (transcriptomics, methylomics, proteomics) (Virant-Klun *et al.*, 2016, Yu *et al.*, 2017, Zhang *et al.*, 2018) as well as from theca and granulosa cells (Fan *et al.*, 2019) will result in better

differentiation protocols and lead to a consensus on the criteria and functional parameters needed to consider using in vitro-derived human oocytes in the clinic.

From ovarian cortex tissue or cells

In vitro matured oocytes from cultured ovarian cortex tissue

In humans, a recent study has described the culture of fresh human ovarian cortex tissue using a multi-step protocol and reported development of unilaminar follicles, albeit with low efficiency, to generate MII oocytes (McLaughlin *et al.*, 2018). However molecular characterization or attempts to fertilize those oocytes have not been reported.

Efforts to optimize the first-step in ovarian cortex tissue culture, by encapsulation of the ovarian cortex tissue in a 3D-scaffold of biomaterial (alginate and polyethylene glycol (PEG)-fibrinogen) to increase the rigidity of the tissue, eventually in combination with IVA (Lerer-Serfaty *et al.*, 2013, Laronda *et al.*, 2014), or integrating (micro)fluidic technology (Liebenthron *et al.*, 2013, Nagashima *et al.*, 2018) have so far not provided significant improvements regarding maturation to MII oocytes *in vitro*.

In vitro matured oocytes from primordial follicles isolated from ovarian cortex tissue

Upon isolation from the ovarian cortex tissue, the cellular connections between the (squamous) granulosa cells and the oocyte in primordial follicles are immediately disrupted. Even in mice, there is no successful protocol to date to culture isolated primordial follicles in vitro to functional MII oocytes (Eppig and O'Brien, 1996, Guzel and Oktem, 2017, Herta *et al.*, 2018). The development of hydrogels (natural or synthetic), to mimic the stiffness and elasticity of the ovary, may facilitate the generation of the physiological niche to allow complete folliculogenesis *in vitro* (Brito *et al.*, 2014, Choi *et al.*, 2014, Shea *et al.*, 2014, Vanacker and Amorim, 2017, Chiti *et al.*, 2018a).

In mice, this approach has allowed the development of fertilizable MII oocytes from primary and secondary follicles (Xu *et al.*, 2006, Mochida *et al.*, 2013). Preliminary data from macaque primary and secondary follicles (Xu *et al.*, 2018a) and human isolated multilayer secondary follicles cultured in 3D-scaffolds (alginate) on low-adhesion plates (Xiao *et al.*, 2015) suggested their ability to reach the MII stage. Functional characterization has not been reported.

In vitro matured oocytes from cells isolated from the ovary

Many studies have focused on the isolation of (primary) cells, other than oocytes, from the adult ovary (such as ovarian surface epithelium, ovarian follicular fluid, follicular aspirates, ovarian stem cells, oogonial stem cells, female germline stem cells, very small embryonic-like stems and ovarian mesenchymal stem cells) and have investigated their potential to differentiate in vitro to oocytes (Ding *et al.*, 2016, Yazdekhasti *et al.*, 2016, Zarate-Garcia *et al.*, 2016, Porras-Gomez and Moreno-Mendoza, 2017, Vanni *et al.*, 2017, Silvestris *et al.*, 2018, Xu *et al.*, 2018a). However promising, there is currently no evidence that those oocyte-like cells have the ability to mature in vitro to cells similar to MII oocytes, with the capacity to be fertilized and develop to a viable blastocyst embryo even in mouse.

From non-ovarian tissue or cells

In vitro matured oocytes from induced pluripotent stem cells (in vitro gametogenesis)

The generation of mature oocytes and healthy offspring from mouse iPSCs (Hayashi *et al.*, 2017) is described above. This protocol required cells from foetal gonads, thus an alternative source of somatic cells to provide the necessary niche is required (Lan *et al.*, 2013, Sepponen *et al.*, 2017).

In vitro matured oocytes from mesenchymal stromal cells

Differentiation to oocyte-like cells in vitro from MSCs isolated from bone marrow, adipose tissue, endometrium, menstrual and peripheral blood or from extraembryonic tissues, such as umbilical

cord blood or amniotic fluid has been attempted (Vanni *et al.*, 2017, Fazeli *et al.*, 2018), but the evidence of oogenesis remains restricted to the expression of several oocyte-specific genes, often not in a physiological combination.

Treatments to prevent gonadotoxic-induced POI

Protection of the ovary against chemotherapy would provide many advantages over current methods for fertility preservation. This subject has recently been comprehensively reviewed (Spears *et al.*, 2019) thus the interested reader is referred there. A summary of the key approaches currently under investigation is shown in Table 12.

Conclusion

It is important to stress that emerging technologies, however promising, need to be followed by rigorous clinical trials, ensuring internationally accepted standards, to demonstrate efficacy and safety before they can be offered as medical treatment. Moreover, a scientific-medical consensus is required regarding safety and functional criteria that needs to be achieved before considering using in vitro-derived human oocytes clinically. In this regard, a societal debate on what emerging technologies may be considered acceptable for human reproductive purposes is recommended.

Although difficult to predict which technologies will prove efficient and safe, improved treatments that could result in less gonadotoxic effects should be the preferred in cancer patients, due to the preventive character, easy implementation in the clinic, low cost, lower number of invasive procedures and the possibility to maintain both reproductive and endocrine functions. However, in the long run and broader application to FP, progress achieving human folliculogenesis in vitro and or improving (or enhancing) systemic ovarian function is necessary, as these technologies may reveal applicable to the broader context of infertility patients and even contribute to conciliate the reproductive ageing of our modern society with women's natural biological clock, revolutionizing the way we reproduce.

Table 12 Approaches for prevention of gonadotoxic-induced POI currently under investigation (Adapted from (Spears *et al.*, 2019).

Protectant	Target action	Species
AMH/MIS	Accelerated primordial follicle (PMF) activation	Mouse
ATM inhibitors: ETP-46464 KU55399	Direct loss of PMFs	Mouse
ATR inhibitors: ETP-46464 AZD6738	Direct loss of PMFs	Mouse
AS101	Accelerated PMF activation	Mouse
Bortezomib	Atresia	Mouse
Ceramide-1-phosphate	Direct loss of PMFs, vascularisation	Mouse
CHK2 inhibitors: BML277 LY2603618 LY2606368	Direct loss of PMFs	Mouse
CK1 inhibitors: MK-8776 CHIR-124 PMF670462 PMF4800567 PMF5006739	Direct loss of PMFs	Mouse
Crocetin	Accelerated PMF activation	Mouse
Dexrazoxane	Atresia	Mouse
Ghrelin	Accelerated PMF activation	Mouse
G-CSF	Atresia, Vascularisation	Mouse
Imatinib	Direct loss of PMFs Atresia	Mouse
Luteinizing Hormone	Direct loss of PMFs Atresia	Mouse
MDR1	Delivery to ovary	Mouse
Melatonin	Accelerated PMF activation	Mouse
Mesna	Atresia	Rat
Mirtazapine	Atresia	Rat
mTORC inhibitors: Everolimus (RAD001) INK128 Rapamycin	Accelerated PMF activation	Mouse
Resveratrol	Atresia	Rat
Sphingosine-1- phosphate	Direct loss of PMFs	Mouse, Rat, Human
Sildenafil Citrate	Atresia	Rat
Tamoxifen	Direct loss of PMFs Inflammation	Rat Human
miRNAs	multiple targets (apoptosis, activation)-	Mouse

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Annex 1: Guideline group

This guideline was developed by the ESHRE Female Fertility Preservation Guideline Development Group (GDG). The GDG included healthcare professionals with expertise in fertility preservation but with different medical background. As such, the guideline group included reproductive endocrinologists, gynaecologist, oncologist, and a psychologist. Two patient representatives joined the guideline group and attended most of the meetings. We aimed for an equal distribution in gender, region and expertise.

Chair of the GDG

Richard Anderson	University of Edinburgh, Edinburgh (UK)

GDG members

Frederic Amant	Academic Medical Centre Amsterdam, Amsterdam (The Netherlands) Antoni van Leeuwenhoek-Netherlands Cancer Institute, Amsterdam Catholic University Leuven, Leuven (Belgium)
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Patient representatives

Sandra Dwek	
Caroline Maslin	

Methodological support

Nathalie Vermeulen	European Society of Human Reproduction and Embryology (Belgium)

Declarations of interest

All members of the guideline development group were asked to declare possible conflicts of interest by means of the disclosure forms (see *ESHRE Manual for Guideline Development*).

	Conflicts of Interest
Richard Anderson	Research Grant Ferring, Roche Diagnostics Consulting fees Roche Speaker's fees IBSA, Roche
Frederic Amant	None declared
Didi Braat	Research Grant Merck Serono, Goodlife
Arianna D'Angelo	None declared
Susana Chuva de Sousa Lopes	None declared
Isabelle Demeestere	Consulting fees ROCHE, speaker's fees Novartis
Lucy Frith	Consulting fees Teva
Matteo Lambertini	Consulting fees Roche, Speaker's fees Roche, Lilly, Theramex, Takeda
Mariana Moura Ramos	Speaker's fees from Merck Sharp and Dohme
Daniela Nogueira	None declared
Kenny Rodriguez-Wallberg	None declared
Sandra Dwek	
Caroline Maslin	
Nathalie Vermeulen	None declared

17 Annex 2: Abbreviations

ABVD	Doxorubicin, bleomycin, vinblastine, dacarbazine
AMH	Anti-Müllerian hormone
ANCA	Antineutrophil cytoplasmic antibody
AYA	Adolescents and young adults
BEACOPP	Cyclophosphamide, doxorubicin, vincristine, bleomycin, etoposide, procarbazine, prednisone
BEP	Bleomycin, etoposide and cisplatin
BOT	Borderline ovarian tumour
BPES	Blepharophimosis, ptosis, and epicanthus inversus syndrome
BRCA	Breast cancer gene
CED	Cyclophosphamide equivalent doses
CHOEP	CHOP plus etoposide
CHOP	Cyclophosphamide, doxorubicin, vincristine, and prednisone
DA	Decision aid
DIE	Doxorubicin isotoxic equivalent
E_2	Estradiol
EBVP	Epirubicin, bleomycin, vinblastine, prednisone
EP	Etoposide and cisplatin
EPOCH-R	Dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab
ER	Estrogen receptor
FOLFOX	5-fluoruracil plus oxaliplatin
FP	Fertility preservation
FSH	Follicle-stimulating hormone
GAHT	gender-affirming hormone treatment
GV	Germinal vesicle
HR	Hazard ratio
HSCT	Haematopoietic stem cell transplantation
Hx	Hysterectomy
IMRT	Intensity-modulated radiotherapy
iPSCs	Induced pluripotent stem cells
IRR	Incidence rate ratio
IVA	In vitro activation
LH	Luteinizing hormone
MAR	Medically assisted reproduction
MII	Metaphase II
MOPP	Mechlorethamine, vincristine, procarbazine, prednisone
MOPP/ABV hybrid	MOPP/doxorubicin, bleomycin, vinblastine
MSCs	Mesenchymal stromal cells
OPU	Oocyte pick-up
OR	Odds ratio
OTC	Ovarian tissue cryopreservation
OTT	Ovarian tissue transplantation
P_4	Progesterone
PR	Prevalence ratio
RA	Rheumatoid arthritis
RR	Relative risk
RSQB	MOPP/doxorubicin, bleomycin, vinblastine
SIR	Standardised incidence ratio
TAC	Transabdominal cerclage
TAYA	Transgender adolescents and young adults
TM	Transgender men
XELOX	Capecitabine plus oxaliplatin

Annex 3: Research recommendations

Patient information provision and support

Studies are needed comparing the effectiveness and patients' satisfaction with paper compared to online decision aids.

The relevance of the decision aids in supporting patients' decision making and reducing emotional distress at the time of the decision should be further clarified.

Studies should investigate the benefit of providing psychological counselling to women undergoing FP decision-making. It should also be investigated which patients would benefit the most from psychological support and counselling. There is a need for more studies examining risk factors for emotional distress in patients undergoing FP.

Gonadotoxicity

To investigate the impact of newer anticancer treatments (including targeted agents and immunotherapy) on ovarian function, ovarian reserve and fertility potential of cancer patients should be considered a research priority.

Oocyte and embryo cryopreservation

The success rates of oocyte versus embryo cryopreservation should be further investigated;

Elective oocyte cryopreservation

Future research: data should be collected on numbers of women who return to use their frozen oocytes and pregnancy and live birth rates. The psychological benefits of having frozen oocytes should also be explored, as fertility could be argued to be preserved even if the oocytes are never used. It could also be explored if better education of both men and women about reproductive lifespan would affect the usage or perceptions of elective oocyte cryopreservation.

Ovarian tissue cryopreservation

- Evaluate the effectiveness of OTC in restoring fertility in larger cohorts of patients.
- Evaluate long-term safety of OTC and replacement for patients and their children (long-term follow-up).
- Studies are needed on graft longevity, and factors affecting this (location of transplantation, surgical technique, follicle density)
- Develop highly sensitive methods for detection of neoplasic cells within the ovarian cortex of high-risk patients

Ovarian protection

Research efforts are needed to provide more evidence on the role of GnRH agonists in ovarian function protection for patients with diseases other than breast cancer. In addition, the collection of long-term follow-up data (including pregnancies and age at menopause) from the already existing randomized trials should be encouraged to provide more robust evidence on the role of this strategy also for fertility preservation. Finally, well-designed and adequately conducted in vitro and in vivo experimental studies should be conducted also in species other than rodents to finally elucidate the protective mechanisms of action of this strategy.

After treatment care

- The effect of pelvic radiotherapy in adults on pregnancy outcomes should be further investigated.
- The follow-up of children after FP treatments should be included in registers.
- Research should investigate the on psychological outcomes of women pregnant after FP.

Annex 4: Methodology

Guideline development

European Society of Human Reproduction and Embryology (ESHRE) guidelines are developed based on the Manual for ESHRE guideline development (N. Vermeulen, A. D'Angelo, P. de Sutter, W.L.D.M. Nelen, Manual for ESHRE guideline development, version 2017), which can be consulted at the ESHRE website (www.eshre.eu/guidelines). The principal aim of this manual is to provide stepwise advice on ESHRE guideline development for members of ESHRE guideline development groups. The manual describes a 12-step procedure for writing clinical management guidelines by the guideline development group, supported by the ESHRE methodological expert.

The current guideline was developed with support of ESHRE, which covered expenses associated with the guideline meetings (travel, hotel and catering expenses) associated with the literature searches (library costs, costs associated with the retrieval of papers) and with the implementation of the guideline (printing, publication costs). Except for reimbursement of their travel expenses, GDG members did not receive any payment for their participation in the guideline development process.

After approval of the guideline application by the ESHRE Executive Committee, the scope of the guideline and the members of the guideline group were discussed by the coordinator and deputies of the ESHRE Special Interest Group (SIG) Safety and Quality in ART and the SIG Fertility Preservation. In composing a guideline group, we strived towards a balance in expertise, gender and location within Europe. A meeting of the guideline development group was organized to discuss the key questions and redefine them through the PICO process (patients – interventions – comparison – outcome). This resulted in a final list of 21 key questions, of which 7 were answered as narrative questions, and 14 ad PICO questions. Based on the defined key words, literature searches were performed by the methodological expert (Dr. N. Vermeulen). Key words were sorted to importance and used for searches in PUBMED/MEDLINE and the Cochrane library. We searched the databases from inception up to 1 November 2019.

Literature searches were performed as an iterative process. In a first step, systematic reviews and meta-analyses were collected. If no results were found, the search was extended to randomized controlled trials, and further to cohort studies and case reports, following the hierarchy of the levels of evidence. Reference were selected or excluded by the methodological expert and expert GDG member based on title and abstract and knowledge of the existing literature. If necessary, additional searches were performed in order to get the final list of papers. The quality of the selected papers was assessed by means of the quality assessment checklist, defined in the ESHRE guideline manual. Next, the evidence was collected and summarized in an evidence table. The quality assessment and completion of evidence tables were performed by the expert GDG members.

Summary of findings tables are usually prepared according to the GRADE approach for all interventions with at least two studies (RCTs) per outcome. For the interventions in the current guideline, such evidence is not available, and hence no summary of findings tables were produced.

GDG meetings were organized to discuss the draft recommendations and the supporting evidence and to reach consensus on the final formulation of the recommendations. In a final step, all evidence and recommendations were combined in the ESHRE guideline: "Female Fertility Preservation"

Formulation of recommendations

We labelled the recommendations as either "strong" or "weak" according to the GRADE approach, with appropriate wording for each option. Suggested interpretation of strong and conditional recommendations by patients, clinicians and health care policy makers is as follows:

Implications for	Strong recommendation	Weak (or conditional) recommendation
Patients	Most individuals in this situation would want the recommended course of action, and only a small proportion would not	The majority of individuals in this situation would want the suggested course of action, but many would not
Clinicians	Most individuals should receive the intervention. Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator. Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences	Recognize that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences Decision aids may be useful in helping individuals to make decisions consistent with their values and preferences
Policy makers	The recommendation can be adopted as policy in most situations	Policy making will require substantial debate and involvement of various stakeholders

For each recommendation, it is mentioned whether it is strong or weak and what the quality of the supporting evidence was. In the justification section, more data are provided on the interpretation of the supporting evidence and how other factors (i.e. balance between desirable and undesirable effects, certainty of the evidence of effects, certainty in how people value the outcome, acceptability and feasibility of the intervention) were considered. Impact on health equity and resource impact were only discussed where relevant.

Strategy for review of the Guideline draft

After finalization of the guideline draft, the review process was initiated. The draft guideline was published on the ESHRE website, accompanied by the reviewers' comments form and a short explanation of the review process. The guideline was open for review between 6 May and 17 June 2020.

To notify interested clinicians, we sent out an invitation to review the guideline by email to all members of the ESHRE SIG Safety and Quality in ART and the SIG Fertility Preservation. Selected reviewers were personally invited by email. These reviewers included:

- Coordinators and deputies of the ESHRE SIGs Embryology, Psychology and Counselling, ….
- Contact persons of patient organizations across Europe.
- Contact persons of international and national societies focused on FP across Europe.

All reviewers are listed in Annex 5. The Reviewer comments processing report, including further information on the review and a list of all comments per reviewer with the response formulated by the GDG is published on the ESHRE website.

Guideline Implementation strategy

The standard dissemination procedure for all ESHRE guidelines comprises publishing and announcement.

Each guideline is published on the ESHRE Website and in Human Reproduction. The announcement procedure includes a newsflash on the ESHRE website homepage. All participants in the annual ESHRE meeting and all related national societies and patient organizations are informed about the guideline release. The latter are asked to encourage local implementation by, for instance, translations or condensed versions, but they are also offered a website link to the original document.

Patient versions of the guideline will be developed by a subgroup of the GDG together with patient the representatives. The patient version is a translation of the recommendations in everyday language, with emphasis on questions important to patients. It aims to help patients understand the guideline's recommendations and facilitates clinical decision-making.

To further enhance implementation of the guideline, the members of the GDG, as experts in the field, will be asked to make suggestions for tailor-made implementation interventions (e.g. option grids, flow-charts, additional recommendations, addition of graphic/visual material to the guideline).

Schedule for updating the guideline

The current guideline will be considered for revision in 2024 (four years after publication). An intermediate search for new evidence will be performed two years after publication, which will inform the GDG of the necessity of an update.

Every care is taken to ensure that this publication is correct in every detail at the time of publication. However, in the event of errors or omissions, corrections will be published in the web version of this document, which is the definitive version at all times. This version can be found at www.eshre.eu/guidelines.

For more details on the methodology of ESHRE guidelines, visit www.eshre.eu/guidelines

Annex 5: Stakeholder review

The guideline draft was published for review for 6 weeks, between 6 May and 17 June 2020. All reviewers, their comments and the reply of the guideline development group are summarized in a review report, which is published on the ESHRE website as supporting documentation to the guideline. The list of representatives of professional organization, and of individual experts that provided comments to the guideline are summarized below.

Annex 6 : Survey on the legal aspects and storage: methodology

A survey was set up in Surveymonkey and distributed to the ESHRE Committee of national representatives (May 2019). There were 33 replies, of which 5 were excluded because only the first question (country) was completed. A second invitation to complete the survey was sent in November 2019 to representatives of countries for which no input was received after the first mailing.

In total, there were 39 replies providing data for 30 countries. There were 3 replies for Italy, and 2 for Belgium, Bulgaria, Croatia, France, Hungary, Russian Federation and UK. For these countries, replies were summarized. Where respondents replied differently, or where information was missing, GDG members or members of the SIG Fertility Preservation coordination were asked to complete the information.