

**Advisory Committee on  
Assisted Reproductive Technology**

**The Use of Cryopreserved Ovarian  
Tissue to Restore Ovarian Function**

Proposed advice  
to the Minister of Health

**Consultation Document**

Citation: Advisory Committee on Assisted Reproductive Technology. 2016. *The Use of Cryopreserved Ovarian Tissue to Restore Ovarian Function: Proposed advice to the Minister of Health*. Wellington: Advisory Committee on Assisted Reproductive Technology.

Published in April 2016 by the Advisory Committee on Assisted Reproductive Technology, PO Box 5013, Wellington 6145, New Zealand

ISBN 978-0-947491-90-1 (online)  
HP 6386

This document is available on the ACART website:  
[www.acart.health.govt.nz](http://www.acart.health.govt.nz)





# Chair's foreword

---

The Advisory Committee on Assisted Reproductive Technology (ACART) has prepared this consultation document to present proposed advice to the Minister of Health that the use of cryopreserved ovarian tissue to restore ovarian function become an established procedure. This means that stored ovarian tissue could be used by fertility clinics to restore a woman's own reproductive function without the need for ethical approval by the Ethics Committee on Assisted Reproductive Technology (ECART).

Human assisted reproductive technologies are continuously developing and rapidly changing. This dynamic is recognised in New Zealand's Human Assisted Reproductive Technology Act 2004 (HART Act). The HART Act seeks to secure the benefits of assisted reproductive technology while protecting the health and safety of all individuals, particularly women and children, in the use of these technologies.

ACART has previously provided advice to the Minister that a procedure or treatment become an established procedure. In 2009, following ACART's advice to the Minister, the use of frozen eggs in fertility treatment became an established procedure.

Ovarian tissue cryopreservation was declared an established procedure in 2005. The subsequent use of the tissue was not, as the safety of its use could not be adequately assessed at the time. ACART is now proposing to advise the Minister that the use of cryopreserved ovarian tissue to restore ovarian function become an established procedure. Currently women in New Zealand are able to cryopreserve and store ovarian tissue, but are unable to use it. ACART has reviewed the recent evidence and considered the risks, benefits and ethical issues associated with the use of cryopreserved ovarian tissue. ACART has concluded that there is good to reason to allow women to use cryopreserved ovarian tissue to restore ovarian function as an established procedure.

We welcome feedback on our proposed advice to the Minister. Your comments will help ACART to finalise its advice to the Minister on the use of cryopreserved ovarian tissue to restore ovarian function.



Alison Douglass

**Chair, Advisory Committee on Assisted Reproductive Technology**

April 2016





# How to have your say

---

Your feedback is important to help ACART finalise its advice to the Minister of Health on the use of cryopreserved ovarian tissue to restore ovarian function.

Please take this opportunity to have your say. A feedback form is at the back of this document. You may give feedback on your own behalf or as a member of an organisation. You can contribute your views by:

1. emailing a completed feedback form or your comments to [acart@moh.govt](mailto:acart@moh.govt), or
2. posting a completed feedback form or your comments to:  
ACART Secretariat  
PO Box 5013  
Wellington.

ACART welcomes your views on any or all of the issues raised.

## ***Publication of feedback on ACART's website***

We will place all feedback on ACART's website as it is received, including your name and contact details, unless you request otherwise by ticking the relevant boxes in the feedback form.

## ***Official Information Act requests — feedback***

Please note that all feedback, including your name and contact details, may be requested by any member of the public under the Official Information Act 1982 (the Act). The Act requires the Ministry of Health (the Ministry) to release your feedback, including your name and contact details, to the person who requested it unless one of the exceptions set out in Part 1 of the Act applies. If there is any part of your feedback that you consider should be withheld under the Act, please make this clear on your feedback form, noting the reasons.

## ***Official Information Act requests — name and contact details***

In accordance with guidance from the Ombudsman, the Ministry's standard procedure is to not release the name and contact details of a submitter who has given feedback in their private capacity (i.e. not in a professional capacity or on behalf of an organisation) and who has requested that the information not be published by ticking the relevant boxes on the feedback form.

Where feedback is given on behalf of an organisation, the Ministry will release the name and contacts details of the submitter and the organisation unless there are other reasons for withholding the information in accordance with the Act. If you consider that your/your organisation's name and/or contact details should be withheld under the Act, please make this clear on your feedback form, noting the reasons.

Further guidance on releasing information under the Act is available at [www.ombudsman.parliament.nz/resources-and-publications](http://www.ombudsman.parliament.nz/resources-and-publications)

**The closing date for feedback is Monday 4 July 2016.**

# Contents

---

Chair’s foreword .....	iii
How to have your say .....	v
Executive summary .....	ix
<b>1 Introduction.....</b>	<b>1</b>
1.1 Purpose.....	1
1.2 Scope of ACART’s proposed advice .....	1
1.3 ACART’s role .....	2
<b>2 Background: ovarian tissue cryopreservation .....</b>	<b>5</b>
2.1 What is ovarian tissue cryopreservation? .....	5
2.2 Ovarian tissue cryopreservation in New Zealand .....	5
2.3 Use of cryopreserved ovarian tissue to restore ovarian function .....	6
2.4 ACART monitoring of the procedure.....	7
<b>3 Assessment of known risks and benefits to health associated with the use of cryopreserved ovarian tissue to restore ovarian function .....</b>	<b>9</b>
3.1 Risks .....	9
3.2 Benefits .....	11
3.3 Monitoring .....	12
<b>4 Acceptability of the risks associated with the use of cryopreserved ovarian tissue to restore ovarian function.....</b>	<b>13</b>
4.1 Indicators of acceptability .....	13
4.2 Relevant principles of the Human Assisted Reproductive Technology Act 2004 .....	16
4.3 ACART’s conclusion .....	17
<b>5 Ethical analysis.....</b>	<b>19</b>
5.1 Principles of the HART Act 2004 .....	19
5.2 Ethical principles from the framework.....	20
5.3 ACART’s conclusion .....	21
<b>6 Conclusion: Proposed advice to the Minister of Health .....</b>	<b>23</b>
Glossary .....	25
<b>Appendix 1: Report on the current status of the use of cryopreserved ovarian and testicular tissue for the Advisory Committee on Assisted Reproductive Technology (ACART) of New Zealand .....</b>	<b>27</b>

<b>Appendix 2: Risk assessment of the use of cryopreserved ovarian tissue to restore ovarian function .....</b>	<b>68</b>
Risk .....	68
Likelihood .....	68
Consequences .....	69
Comparing the risks .....	71
<b>Feedback form.....</b>	<b>73</b>
<b>Questions for response .....</b>	<b>74</b>
Question 1 .....	74
Question 2 .....	75
Question 3 .....	76
Question 4 .....	77
Question 5 .....	78
Question 6 .....	78
Question 7 .....	79





# Executive summary

---

In 2005, storage of cryopreserved ovarian tissue was declared an established procedure. This means it can be routinely offered by fertility clinics and does not require case by case ethical review by the Ethics Committee on Assisted Reproductive Technology (ECART). The subsequent use of cryopreserved ovarian tissue to restore ovarian function was not included in the established procedure because of the novelty of the procedure and limited evidence available at the time.

Women in New Zealand therefore may cryopreserve and store ovarian tissue, but are unable to use it in New Zealand. There are now good reasons to allow women to use their own cryopreserved ovarian tissue to restore ovarian function and increase the possibility of reinstating reproductive function.

The Advisory Committee on Assisted Reproductive Technology (ACART) has reviewed recent evidence and considered the risks, benefits and ethical issues associated with this use of cryopreserved ovarian tissue.

ACART proposes advising the Minister of Health that the use of cryopreserved ovarian tissue to restore ovarian function in the woman from whom the tissue was excised should become an established procedure for the following reasons:

- There have been significant advances, and an increasing uptake in the use of cryopreserved ovarian tissue for restoring fertility and ovarian function in women.
- Ovarian tissue cryopreservation is currently an established procedure and with fertility preservation becoming an integral part of cancer treatment, there is likely to be increasing demand to use stored tissue.
- Women with grafted ovarian tissue do not appear to experience a relapse in cancer more often than women who have not undergone an ovarian tissue transplant.
- Women in New Zealand with stored ovarian tissue may travel overseas where ovarian tissue transplantation is permissible, for example to Australia. It would be preferable for women to receive treatment in New Zealand.
- The available evidence suggests that there are no reported risks to the resulting children born from cryopreserved ovarian tissue compared to children conceived naturally or through other methods of assisted reproductive technologies.
- There are no significant ethical issues associated with the use of cryopreserved ovarian tissue to restore ovarian function.

If the Minister approves the use of cryopreserved ovarian tissue to restore ovarian function as an established procedure, ACART will keep a watching brief on international developments and reported findings in the use of cryopreserved ovarian tissue.





# 1 Introduction

---

## 1.1 Purpose

1. Ovarian tissue cryopreservation is an established procedure but the subsequent use of cryopreserved ovarian tissue is not.
2. This consultation document invites public comment on the proposed advice from the Advisory Committee on Assisted Reproductive Technology (ACART) to the Minister of Health (the Minister) that the use of cryopreserved ovarian tissue to restore ovarian function in the woman from whom the tissue was excised, for her own treatment, become an established procedure.

## 1.2 Scope of ACART's proposed advice

### 1.2.1 *In scope*

3. ACART's proposed advice focuses on the use of cryopreserved ovarian tissue by the woman from whom the tissue was excised, for her own treatment, to restore ovarian function.

### **Restoring ovarian function**

4. The aim of restoring ovarian function is to restore hormonal activity and production of eggs suitable for fertilisation. The primary intention for restoring ovarian function is to provide a potential option for restoring a woman's fertility. In addition to restoring hormonal activity and producing eggs, other physiological processes are interrelated with ovarian function. For example, preventing early onset of osteoporosis and cardiovascular disease in young women, enabling pre-pubescent girls to undergo puberty at the appropriate age, preventing early onset menopause, and reducing hormone therapies following gonadotoxic treatment.

### 1.2.2 *Out of scope*

5. The use of frozen eggs and embryos are considered out of scope because these are already established procedures.

### **Donation of cryopreserved ovarian tissue to another individual**

6. ACART is not proposing that the donation of cryopreserved ovarian tissue to another individual should become an established procedure. Based on current evidence, ACART does not support the transplantation of cryopreserved ovarian tissue to another individual. The potential benefits to be gained are outweighed by the

uncertainties and potential risks to the recipient, including rejection of the transplanted tissue or the possible transfer of malignant cells and disease.

### **Use of cryopreserved ovarian tissue for research purposes**

7. Women may decide they do not wish or need to use their cryopreserved ovarian tissue. In this case, they may choose to discard the tissue or donate it for research purposes. Where the subsequent use of stored ovarian tissue is intended for research, this falls within the scope of human reproductive research. ACART is not making any proposal in regards to the use of surplus cryopreserved ovarian tissue in human reproductive research.
8. Any research proposal involving the use of donated ovarian tissue is covered by ACART's *Guidelines for Research on Gametes and Non-Viable Embryos* and will require ECART approval.<sup>1</sup>
9. This situation is comparable to individuals with stored eggs or sperm. If there are eggs or sperm that they do not intend to use, they have the option to donate to others, discard, or donate to research. However, in light of the current evidence, ACART considers that cryopreserved ovarian tissue should not be donated to another individual for their therapeutic use.

## **1.3 ACART's role**

10. ACART's role under the Human Assisted Reproductive Technology Act 2004 (HART Act 2004) is to:
  - issue guidelines and advice to the Ethics Committee on Assisted Reproductive Technology (ECART) on any matter relating to any kind of assisted reproductive procedure, human reproductive research, and extended storage of gametes and embryos
  - provide the Minister with advice on aspects of, or issues arising out of, different kinds of assisted reproductive procedures or human reproductive research
  - monitor the application and health outcomes of assisted reproductive procedures and established procedures and developments in human reproductive research.
11. The HART Act 2004 enables ACART to recommend that the use of cryopreserved ovarian tissue to restore ovarian function be one of the following:
  - an established procedure
  - subject to ethical approval on a case-by-case basis (therefore requiring guidelines)
  - subject to a moratorium
  - prohibited.

---

<sup>1</sup> Section 16, HART Act 2004.

12. An established procedure is a procedure that is declared established under section 6 of the HART Act 2004 and that can be routinely undertaken by fertility clinics, without the clinic having to seek ethical approval from ECART on a case-by-case basis.
13. In giving advice to the Minister, ACART is required by the HART Act 2004 to provide the Minister with a report that sets out:
  - information about the procedure or treatment
  - an assessment, drawn from published and peer-reviewed research, of the known risks and benefits to health of the procedure or treatment
  - advice on whether, in its expert opinion, the known risks to health resulting from the procedure or treatment fall within a level of risk that is acceptable in New Zealand
  - an ethical analysis of the procedure or treatment
  - advice on whether, in its expert opinion, the Minister should recommend that the procedure or treatment be declared an established procedure.
14. This document follows the above format.





## 2 Background: ovarian tissue cryopreservation

---

### 2.1 What is ovarian tissue cryopreservation?

15. Ovarian tissue cryopreservation is a technology that has been developed for preserving reproductive potential in girls and women with medical conditions such as cancer. Women who undergo cancer treatments, such as chemotherapy or radiotherapy, may experience adverse consequences to their fertility, for example early onset of menopause. Cancer treatments, such as chemotherapy and radiotherapy, may have potentially gonadotoxic effects causing menopause or adverse consequences for her fertility.
16. Ovarian tissue cryopreservation has been used since the late 1990s. Currently, the ovarian tissue cryopreservation procedure is most commonly used in young girls (ie. prepubertal) and women facing the possible loss of their fertility from cancer treatment.
17. It is sometimes also used by young girls and women with a genetic disease, or non-malignant disease where treatment may adversely affect their fertility.

### 2.2 Ovarian tissue cryopreservation in New Zealand

18. In 2005 ovarian tissue cryopreservation was declared an established procedure under the HART Order 2005.
19. As part of implementing the HART Act 2004, and before ACART was established, an independent committee, the Advisory Group on Assisted Reproductive Technologies (the Advisory Group) advised the Director-General of Health on the risks and benefits of various assisted reproductive technologies. The Advisory Group recommended that ovarian tissue cryopreservation become an established procedure. The Advisory Group considered that the risks of ovarian tissue cryopreservation were those associated with the surgery to retrieve the tissue, and were therefore minimal. The procedure offered women and girls about to undergo cancer treatment the opportunity to retain their reproductive potential.
20. The Advisory Group's recommendation was accepted. The established procedure includes:
  - collecting and preparing ovarian tissue for freezing,
  - freezing and storing the tissue, and
  - thawing and discarding the tissue.

21. In its 2005 report, the Advisory Group did not recommend that the subsequent use of cryopreserved ovarian tissue should become an established procedure. The Advisory Group advised that the safety of such use was in doubt due to the novelty of the procedure and limited evidence available at the time.
22. An effect of excluding the use of cryopreserved ovarian tissue from the established procedure is that ECART must approve the use of the tissue. To date ACART has not issued any guidelines or advice on the use of cryopreserved ovarian tissue, therefore ECART has not been able to consider any applications on the use of cryopreserved ovarian tissue.
23. As a result, women in New Zealand may cryopreserve and store ovarian tissue, but are unable to use it.

## 2.3 Use of cryopreserved ovarian tissue to restore ovarian function

24. Once ovarian tissue has been cryopreserved it may be thawed and transplanted into the woman from whom it was excised.
25. The primary use of cryopreserved ovarian tissue is to restore ovarian function. The aim of restoring ovarian function is to restore production of ovarian hormones and eggs suitable for fertilisation. These contribute to restoring a woman's potential fertility.
26. Fertility has been restored following transplantation of the tissue in two different situations – transplantation to the original site of the ovaries and/or transplantation to a site outside of the ovaries.
27. In most European countries, cryopreserved ovarian tissue must only be used by the woman from whom it originated. It is illegal to treat another person with the tissue.
28. It is estimated more than 10,000 women internationally have stored cryopreserved ovarian tissue for fertility purposes, and an estimated 200 women have received transplantation worldwide. There is growing awareness that in addition to providing gametes, transplanted tissue may serve an important endocrine function for the woman.



## 2.4 ACART monitoring of the procedure

29. ACART has kept a watching brief on developments in the use of cryopreserved ovarian tissue. In 2010 the Committee commissioned a technical report to review the use of cryopreserved ovarian tissue and obtain further information.<sup>2</sup> ACART at the time concluded that the limited evidence did not support further work to enable the use of the tissue in New Zealand.
30. In 2014 ACART commissioned a further technical report 'Report on the current status of the use of cryopreserved ovarian and testicular tissue for the Advisory Committee on Assisted Reproductive Technology of New Zealand' by Professor Claus Andersen of Oslo University.<sup>3</sup> Based on the findings, ACART agreed to develop advice to the Minister that the use of cryopreserved ovarian tissue to restore ovarian function should become an established procedure.

---

<sup>2</sup> Anderson R. 2010. Report on the Current Status of the Use of Cryopreserved Ovarian Tissue. URL: [www.acart.health.govt.nz/report-current-status-use-cryopreserved-ovarian-tissue-2010](http://www.acart.health.govt.nz/report-current-status-use-cryopreserved-ovarian-tissue-2010)

<sup>3</sup> Professor Claus Yding Andersen is a Professor of Human Reproductive Physiology at the University of Copenhagen. He was commissioned to produce the technical report due to his significant and recent expertise in both ovarian and testicular cryopreserved tissue, including safety and outcomes. He is a leading fertility treatment clinician and has headed the Danish programme on fertility preservation since it began in 1999.



# 3 Assessment of known risks and benefits to health associated with the use of cryopreserved ovarian tissue to restore ovarian function

---

31. This section summarises the known risks and benefits associated with the use of cryopreserved ovarian tissue to restore ovarian function as discussed in the 'Report on the current status of the use of cryopreserved ovarian and testicular tissue for the Advisory Committee on Assisted Reproductive Technology of New Zealand'. The report is included in full at Appendix 1.<sup>4</sup>

## 3.1 Risks

### 3.1.1 *Risks to the tissue*

32. There are no reports that suggest the cryopreservation procedure itself imposes any change in the tissue and could lead to unwanted side effects upon transplantation. In addition, there are no reports on any potential risks to a woman's health through the use of cryopreserved ovarian tissue in terms of oocytes and subsequent embryos being abnormal or less viable as compared to mature oocytes obtained in in vitro fertilisation (IVF) treatment.
33. There is a risk of damage to oocytes and follicles in the ovarian tissue during the cryopreservation procedure. Data suggests that women who undergo IVF treatment following transplantation of thawed tissue have lower rates in oocyte retrieval rate and embryo formation. Consequently, this has an effect on pregnancy rates.
34. Currently it is not possible to predict who will conceive, and who will not, from their transplanted tissue. This in turn has an effect on understanding pregnancy rates.
35. It is not yet possible to detect the number of follicles present without destroying the tissue in the process, nor is there solid information of the survival rate of follicles following transplantation to a woman. The chances of conception will in part depend on the age of the woman at the time of tissue cryopreservation. The older the woman, and therefore her ovarian tissue, contributes to a lower chance of conceiving. In

---

<sup>4</sup> Note that the technical report discusses the use of both cryopreserved ovarian and testicular tissue. In this proposed advice, ACART is addressing the use of cryopreserved ovarian tissue. The use of cryopreserved testicular tissue will be considered in the future as a separate project when sufficient international studies have indicated the utility and safety of this tissue.

addition to age, the collected amount of tissue may correspond to the number of follicles that could develop, and affect the subsequent success of the treatment.

### *3.1.2 Outcomes for children born from cryopreserved ovarian tissue*

36. Currently more than 35 children have been born in eight countries from transplanted cryopreserved ovarian tissue. All babies born have been reported to be healthy. There is no information to suggest children born from cryopreserved ovarian tissue are any different from children conceived naturally.
37. At this time, there is no systematic reporting of pregnancy and birth data. Consequently, this limits the information available in terms of pregnancy outcomes, and has implications on the monitoring and assessment of longer-term health outcomes on babies born from cryopreserved ovarian tissue.
38. There are also no long term follow-up studies on the children and their development at present. It is too early to identify if there are any adverse observations of a child's development or psychological outcomes, given the current ages of children born to date. There is insufficient information on resultant chromosomal abnormalities or congenital malformations or epigenetic disorders. Current evidence about children conceived from frozen eggs, indicates no deleterious effects of the cryopreservation process.
39. Any trans-generation effects on the children born from mothers who become pregnant from transplantation are unknown.
40. ACART concludes that the evidence at this stage suggests that health outcomes for children born from the use of cryopreserved ovarian tissue are similar to those for children born as a result of other IVF procedures including frozen eggs. ACART, however recognises that further research is needed in this area.

### *3.1.3 Maternal health outcomes*

#### **Concerns with transplanting tissue excised at the time of disease**

41. The safety of grafting ovarian tissue excised at the time when the patient experienced active disease is currently unresolved. There is a potential risk of malignant cells from the original cancer being present in the transplanted tissue. If the tissue was excised at the time of active disease, the transplanted tissue may cause a relapse. There is currently no way to detect with certainty, malignant cell contamination in the ovarian tissue. Nor is it known how many grafted cancer cells would cause a relapse.
42. To date, women with grafted ovarian tissue do not appear to experience relapse more often than that of cancer survivors without transplanted ovarian tissue. In Denmark, 15 women who have had their cryopreserved ovarian tissue transplanted for more than five years have not shown signs of relapse following their transplantation.

43. Based on an estimated 200 cases of transplanted ovarian tissue worldwide, there have been six reported cases of a woman experiencing relapse following transplantation. Approximately 120–150 women had cancer at the time of tissue retrieval. The reported tumour relapses occurred at the original site of the tumour and there has been no indication of the ovarian tissue causing relapse. A recent study of 41 Danish women with transplanted ovarian tissue reported three cases of relapse but all cases appear to be unrelated to the transplant.<sup>5</sup>
44. Leukaemia is usually considered the highest risk for relapse in connection with grafting because leukaemia is a disease of the blood, and malignant cells will almost invariably be present in the tissue. The literature recommends that collection of ovarian tissue from leukaemia patients occur when they are in complete remission.

### **Pregnancy-related risk**

45. There are no known higher pregnancy-related risks for women with transplanted ovarian tissue. The only risk is that their cancer treatment may have affected their ability to carry a pregnancy, for example damage from irradiation of the pelvis.

### **Surgical risk**

46. There are no reported increased procedural risks with transplanting thawed ovarian tissue. There is, however a risk with any surgical procedure, for example, infection, bleeding or damage to adjacent organs.
47. The procedure of removing and replacing ovarian tissue is considered low risk, and no major complications from the operation have been reported.

## **3.2 Benefits**

48. The use of cryopreserved ovarian tissue to restore ovarian function has made significant advances. It is gaining ground as a valid method for restoring fertility and ovarian activity more generally. Benefits from the use of cryopreserved ovarian tissue include:
  - This may be the only opportunity for some girls and women undergoing gonadotoxic treatment to regain their fertility in the future.
  - It has the potential to increase quality of life following cancer treatment because fertility is valued by many girls and women.
  - Ovarian function can be restored in a woman for years at a time following reimplantation. One study reported this to be the case for 93% of patients with transplanted ovarian tissue. Another study in Denmark reported 18 women have so far regained, and continue to maintain, their ovarian function for at least seven to nine years. Restoring ovarian function includes the return of endocrine function,

---

<sup>5</sup> Jensen AK, Kristensen SG, Macklon KT, et al. 2015. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. *Human Reproduction* 30 2838–45.

menstrual cycles, production of eggs suitable for fertilisation, and the possibility of pregnancy – both assisted and spontaneous.

- For younger women, regaining their menstrual cycles is not only consistent with restoring their potential fertility, but it would help delay the onset of osteoporosis and other menopausal related conditions.
- An ovarian tissue transplant can allow for a pregnancy to occur as long as the tissue remains active.

49. These benefits are only possible if the subsequent use of cryopreserved ovarian tissue is permitted.

### 3.3 Monitoring

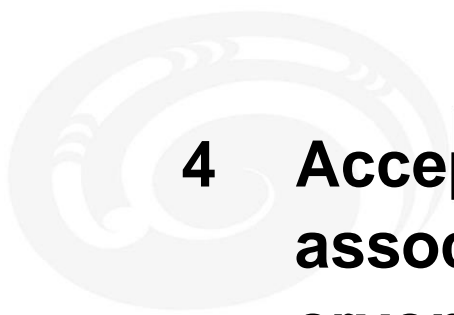
50. The HART Act 2004 requires ACART to monitor the application and health outcomes of assisted reproductive procedures and established procedures, and also to monitor developments in human reproductive research.<sup>6</sup>

51. The lack of evidence discussed above highlights the need for ACART to keep a watching brief on international developments and reported findings in the use of cryopreserved ovarian tissue to restore ovarian function, particularly regarding outcomes for children born from the procedure and health outcomes for women.

52. ACART notes that it will be difficult to collect data on the outcomes for the use of cryopreserved ovarian tissue to restore ovarian function. For example, when collecting data on pregnancy rates, a pregnancy may occur spontaneously independent of the graft itself; the graft may fail; the cryopreserved tissue may never be used; or there may be an extended length of time from the time of grafting until the woman becomes pregnant. Notwithstanding the difficulty of data collection, ACART would support the establishment of an international database to monitor both cryopreserved ovarian tissue transplants and outcomes for children born from transplanted tissue.

---

<sup>6</sup> Section 35(2) HART Act 2004.



## 4 Acceptability of the risks associated with the use of cryopreserved ovarian tissue to restore ovarian function

---

53. This section presents ACART's analysis on the acceptability of the risks associated with the use of cryopreserved ovarian tissue to restore ovarian function.
54. ACART has applied its risk acceptability framework to consider and assess the acceptability of the risks associated with the use of cryopreserved ovarian tissue to restore ovarian function (see attached Appendix 2).
55. As discussed in section 3 of this document, ACART has identified very few known health risks associated solely with the use of cryopreserved ovarian tissue to restore ovarian function. ACART's analysis indicates that these fall within a level of risk that is acceptable in New Zealand.

### 4.1 Indicators of acceptability

56. There are very few known risks in the use of cryopreserved ovarian tissue that are similar to those of other, more common, assisted reproductive technologies.
57. ACART's assessment of the acceptability of the risks includes:
  - international acceptability of the procedure
  - the potential demand for the use of cryopreserved ovarian tissue in New Zealand
  - who should make decisions about the use of the procedure
  - risk reduction and management
  - benefits.
58. In its analysis of the use of cryopreserved ovarian tissue to restore ovarian function, ACART has considered the amount of stored ovarian tissue, and the views on fertility preservation for cancer patients.

#### 4.1.1 Overseas uptake

59. ACART considers the increasing uptake in other countries as an indication of a worldwide acceptability of the risks associated with the technology. Twenty-six

countries are currently reported to use cryopreserved ovarian tissue.<sup>7</sup> In particular, ACART notes that the Australian and United Kingdom regulatory frameworks for assisted reproduction allows for the transplantation of cryopreserved ovarian tissue.

60. Some countries require ethical approval of the procedure, but no country has banned the procedure except for Bulgaria, where there is a moratorium until formal regulations have been approved.
61. More than 10,000 women have had ovarian tissue cryopreserved worldwide, and of these, an estimated 200 women have received a subsequent ovarian tissue transplant. Recent reports indicate that more than 35 babies worldwide have been born from the use of cryopreserved ovarian tissue.<sup>8</sup>
62. The uptake for ovarian tissue cryopreservation and subsequent transplantation is significantly lower than other assisted reproductive technologies used for fertility preservation. The first choice for fertility preservation of cancer patients is egg freezing when there is enough time to undergo egg collection before beginning cancer treatment.<sup>9</sup>

#### 4.1.2 Potential demand in New Zealand

63. Fertility preservation is rapidly becoming an integral part of cancer patients' treatment, particularly as survival rates for cancer increase. ACART recognises that ovarian tissue cryopreservation as a method of fertility preservation will increase. It therefore follows that there will be an increasing demand for women to be able to take advantage of their stored tissue in order to try and have children. In 2015, a New Zealand fertility clinic reported having ovarian tissue samples stored for 67 women.
64. At present, women in New Zealand who wish to have their cryopreserved ovarian tissue transplanted need to travel overseas, for example, to Australia. ACART is of the view that it is preferable for women to use this procedure in New Zealand because of proximity to family, other support networks, clinical supervision and support.

#### 4.1.3 Decision-maker

65. ACART's analysis is that the identified risks and ethical issues are acceptable, and would not require ethical oversight by ECART. ACART considers that the risks and ethical issues could be addressed in discussion between a patient and her clinician.
66. Section 5 of this document sets out a detailed ethical analysis of the procedure.

---

<sup>7</sup> Denmark, Norway, United Kingdom, Israel, Germany, Switzerland, Sweden, Finland, Holland, Belgium, France, Spain, Italy, United States, Austria, Australia, Japan, Portugal, Poland, Hungary, Singapore, South Africa, Vietnam, Russia, China, India.

<sup>8</sup> Jensen AK, Kristensen SG, Macklon KT, et al. 2015. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. *Human Reproduction* 30: 2838–45.

<sup>9</sup> Kawamura K, Cheng Y, Sun Y, et al. 2015. Ovary transplantation: to activate or not to activate. *Human Reproduction* 30: 2457–60.



#### 4.1.4 Risk reduction and management

67. There are some concerns about the use of cryopreserved ovarian tissue excised at the time of active disease. Overall, the risk of malignant cells from the original cancer being present in the transplanted tissue cannot be excluded, but is considered to be very low. To date, animal and primate studies suggest the development of malignant cells following the transplant of malignant cells, may be considerably lower than anticipated and dependent on the number of malignant cells being transplanted. The number of women to date who have experienced relapse is reassuringly low, and the relapse is most likely unrelated to the ovarian tissue in these cases.
68. ACART has identified precautionary measures undertaken overseas to reduce the risk of transplanting malignant cells. One example is with leukaemia patients. Leukaemia is considered to have the highest risk of causing relapse in connection with grafting, and therefore ovarian tissue may be collected from women with leukaemia when they are in complete remission. In Denmark, transplantation of cryopreserved ovarian tissue is not undertaken in leukaemia patients.
69. Furthermore, transplanting tissue is usually considered only when a woman is well and has been disease-free for at least two years.
70. In light of this, ACART considers the risk of malignant cells being present in the transplanted tissue to be acceptable.

#### 4.1.5 Benefits

71. Overall, ACART considers that the benefits associated with the use of cryopreserved ovarian tissue to restore ovarian function are significant and outweigh the risks.
72. The primary benefit associated with the use of cryopreserved ovarian tissue to restore ovarian function is no different to that of other assisted reproductive technologies such as IVF, or the use of frozen eggs; it is a potential opportunity for a woman to conceive and give birth to a child.
73. A distinguishing benefit of the use of cryopreserved ovarian tissue to restore ovarian function is that it may be the only opportunity for restoring ovarian function and fertility in women who have had potentially gonadotoxic cancer treatment. As ovarian tissue can be cryopreserved on short notice, it can be preferable to egg freezing if there is urgency to commence cancer treatment.
74. Additional benefits include its:
  - potential for a pregnancy to occur as long as the ovarian tissue remains active
  - restoring ovarian function in women
  - establishing ovarian function in prepubescent girls when they reach puberty.
75. These benefits make the risks associated with the technology more acceptable.

#### 4.1.6 *Effect of data uncertainty*

76. In ACART's risk acceptability assessment, the following areas of uncertainty have been identified:
- There are no long term follow-up developmental studies of babies born from transplanted cryopreserved ovarian tissue currently available.
  - There is limited information and data, on pregnancies and births.
  - The success rate of transplanted tissue is unknown, and will only be known once a large cohort of women have used their cryopreserved ovarian tissue.
  - There is a need for information on potential negative consequences or adverse health outcomes of girls and women following transplantation.
  - The safety of the tissue, especially in connection with leukaemia and tumours originating in the ovary itself, remains unknown. There are no studies to confirm that transplanted tissue does not develop into malignancy later on.
  - Excluding the potential risk of malignant cells from the original cancer disease being present in the transplanted tissue, there is no information to suggest that the transplanted tissue should increase the risk of cancer or lead to adverse side effects.
77. ACART will continue to monitor international developments associated with the use of cryopreserved ovarian tissue to restore ovarian function.

## 4.2 Relevant principles of the Human Assisted Reproductive Technology Act 2004

78. ACART is guided in its decision-making by the principles of the HART Act 2004. These are listed below:
- The health and wellbeing of children born as a result of the performance of an assisted reproductive procedure or an established procedure should be an important consideration in all decisions about that procedure.
  - The human health, safety and dignity of present and future generations should be preserved and promoted.
  - Although all persons are affected by assisted reproductive procedures and established procedures, women, more than men, are directly and significantly affected by their application, and the health and wellbeing of women must be protected in the use of these procedures.
  - No assisted reproductive procedure should be performed on an individual, and no human reproductive research should be conducted on an individual, unless the individual has made an informed choice and given informed consent.
  - Donor offspring should be made aware of their genetic origins and be able to access information about those origins.

- The needs, values and beliefs of Māori should be considered and treated with respect.
  - The different ethical, spiritual and cultural perspectives in society should be considered and treated with respect.
79. All of these principles are relevant to a risk-acceptability analysis. These are also discussed in section 5 of the discussion document ‘Ethical analysis’.
80. ACART considers that the use of cryopreserved ovarian tissue to restore ovarian function is consistent with the purposes and principles of the HART Act 2004.
81. In particular, ACART has considered the health and wellbeing of children born as a result of the use of cryopreserved tissue and considers that, at this stage, the evidence does not indicate there are any adverse implications to a child’s development or psychological outcomes.
82. Allowing the use of cryopreserved ovarian tissue to restore ovarian function ensures that women who face gonadotoxic treatment are able to preserve their fertility by having the opportunity to produce oocytes in their future, thereby protecting their health and wellbeing.

### 4.3 ACART’s conclusion

83. ACART considers the increasing uptake of and demand for the use of cryopreserved ovarian tissue overseas indicates the international acceptability of the risks associated with this procedure. This supports ACART’s analysis of the level of risk as acceptable in New Zealand. ACART also considers that the known risks, such as maternal or child health outcomes, do not justify case by case ethical review by ECART, and could be addressed in discussion between a patient and her clinician.
84. ACART acknowledges that while there are known risks and areas of uncertainty, there are risk reduction measures to help ensure these remain at an acceptable level in New Zealand. ACART will maintain a watching brief for developments in the procedure to better understand areas of uncertainty. The literature will indicate clinical practices that may help reduce the risk of transplanting malignant cells into women.
85. ACART is of the opinion that while there are risks associated with the use of cryopreserved ovarian tissue to restore ovarian function (see section 3) these fall within a level of risk that is acceptable in New Zealand.

#### **Feedback questions (see page 74 to 76)**

##### Question 1

- (a) Do you agree with ACART’s assessment of the known risks and benefits to health associated with the use of cryopreserved ovarian tissue to restore ovarian function?

- (b) Are there any risks and/or benefits associated with the use of cryopreserved ovarian tissue to restore ovarian function that ACART has not identified or assessed?

Question 2

- (a) Do you agree with ACART's conclusion that the risks associated with the use of cryopreserved ovarian tissue to restore ovarian function fall within a level that is acceptable in New Zealand?
- (b) Do you have any other comments?

Question 3

- (a) Has ACART identified all the relevant areas to monitor the use of cryopreserved ovarian tissue to restore ovarian function?
- (b) If not, what else should ACART monitor?

## 5 Ethical analysis

---

86. ACART must identify and consider ethical issues that arise from assisted reproductive treatment. ACART is guided by its ethical framework,<sup>10</sup> which incorporates principles of the HART Act 2004 and generally accepted ethical principles, to make these ethical deliberations.

### 5.1 Principles of the HART Act 2004

87. ACART's ethical analysis is guided by the principles of the HART Act 2004.<sup>11</sup> Overall, ACART has not identified any ethical issues that are inconsistent with the health and wellbeing of children; human health, safety and dignity; donation and donor offspring; or the health and wellbeing of women. While recognising that there are unconfirmed implications and data uncertainties for the health and wellbeing of women and children born, ACART considers that the use of cryopreserved ovarian tissue to restore ovarian function is consistent with the ethical principles of the HART Act 2004.

#### 5.1.1 *Informed consent*

88. The HART Act 2004 requires that no assisted reproductive procedure be performed on an individual unless the individual has made an informed choice and given informed consent. This requirement is supported by the Code of Health and Disability Services Consumers Rights, and the Fertility Services Standard which sets out specific informed consent requirements for assisted reproductive technology.
89. Any woman seeking to use cryopreserved ovarian tissue to restore ovarian function must be provided with full information about the procedure to enable them to make an informed choice. ACART is of the view that a woman seeking the procedure should be able to make an informed decision through discussions with her clinician. This would include detailing the components of the procedure, taking into the account the merits and risks, and understanding the areas of uncertainties associated with this procedure – for example, the limited evidence about health outcomes for children born from cryopreserved ovarian tissue. Other information such as the best transfer technique and the size of the tissue transplanted would vary from patient to patient.

#### 5.1.2 *Respect for the needs, values and beliefs of Māori*

90. Principle 4(f) of the HART Act 2004 requires that the needs, values and beliefs of Māori should be considered and treated with respect. This does not mean that ACART supports the idea that a pan-Māori perspective exists. There is rarely one single

---

<sup>10</sup> Ethical framework for ACART (2012)

<http://acart.health.govt.nz/publications-and-resources/acart-publications/ethical-framework-acart>

<sup>11</sup> Section 4 HART Act 2004.

viewpoint representative of Māori concerns, any more than there is a single religious viewpoint.

91. ACART acknowledges the primary significance of whakapapa and the impact on broader family networks associated with the use of assisted reproductive treatment. There may be concern that whakapapa could be disrupted through such treatment. In relation to the use of cryopreserved ovarian tissue to restore ovarian function, ACART notes this procedure may assist Māori women facing limited fertility treatment options, to both preserve and strengthen their whakapapa, because it offers an opportunity to have genetically-related children.
92. The woman must be able to maintain the mana of her cryopreserved ovarian tissue. In this context, ACART acknowledges the concepts of whakapapa, whanaungatanga and kaitiakitanga in relation to the storage and use of cryopreserved ovarian tissue. Regardless of the length of time between collection, storage and subsequent use of the cryopreserved tissue, there must be sufficient time to discuss any cultural implications and the opportunity to address restoration of her ovarian function and fertility.

## 5.2 Ethical principles from the framework

93. These principles are consistent with and complementary to the principles of the HART Act 2004. They include consideration of the welfare of those affected by the procedure, and the autonomy of those involved, altruism, social trust and responsibility, the special status of the embryo, justice and equality.

### 5.2.1 *Welfare and autonomy*

94. ACART's proposed advice that the use of cryopreserved ovarian tissue to restore ovarian function become an established procedure supports a woman's existing freedom to choose from the fertility preservation options available to her. ACART is of the view that women can consider the benefits and risks to decide whether this procedure is in their best therapeutic interest.

### 5.2.2 *Justice and equality*

95. It would be unfair to further restrict the choices of people who face limited options for trying to have a child.
96. While ACART's functions do not involve responsibility for the costs of reproduction, ACART acknowledges that there may be concerns about access to the technology in terms of costs restricting who may be able to access it. Costs would likely include ovarian tissue collection and freezing, storage of tissue and transplantation of thawed tissue. However, ACART considers that the issue of cost for this procedure is no different from the issue of cost and access to other assisted reproductive treatments.

97. ACART considers that the use of cryopreserved ovarian tissue to restore ovarian function is suitable as a potential fertility preservation option for women. However, ACART recommends the use of cryopreserved ovarian tissue be restricted to the woman from whom the tissue was excised for her own treatment. This position is taken in most European countries where this procedure is permissible.

### 5.3 ACART's conclusion

98. ACART is of the view that the ethical issues identified and considered do not justify restricting the use of cryopreserved ovarian tissue to restore ovarian function. The potential benefits to be gained are outweighed by the ethical issues identified.

#### **Feedback questions (see page 77)**

##### Question 4

- (a) Has ACART identified all the ethical issues relevant to the use of cryopreserved ovarian tissue to restore ovarian function?
- (b) Do you agree with ACART's ethical analysis that there are no significant ethical issues associated with the use of cryopreserved ovarian tissue to restore ovarian function?





## 6 Conclusion: Proposed advice to the Minister of Health

---

99. ACART proposes advising the Minister that the use of cryopreserved ovarian tissue to restore ovarian function become an established procedure by the woman from whom the tissue was excised, for her own treatment.
100. ACART notes that a woman may choose to donate stored cryopreserved ovarian tissue for research purposes. However the subsequent use of ovarian tissue for human reproductive research will require further ECART approval.
101. ACART's reasons for its recommendations are as follows:
- There have been significant advances in the use of cryopreserved ovarian tissue to restore ovarian function, and therefore fertility potential, as indicated by international publications.
  - There has been a substantial increase worldwide in the number of cases since 2010 in the use of cryopreserved ovarian tissue. It is estimated that internationally more than 10,000 women have had ovarian tissue cryopreserved and that an estimated 200 women have received a transplantation.
  - Ovarian tissue cryopreservation is currently an established procedure in New Zealand and there is good reason to allow women to subsequently use their stored tissue.
  - The increases in survival for many cancer patients, particularly for children, coupled with the increased focus on fertility preservation mean that many women will want to take advantage of their stored tissue in the future to have children.
  - Women with grafted ovarian tissue do not appear to experience a relapse in cancer more often than women who have not undergone the procedure.
  - Although further studies are required, the available evidence suggests that there are no reported risks to the resulting children born from cryopreserved ovarian tissue compared to children conceived naturally or through other methods of assisted reproductive technologies.
  - There are few ethical issues associated with the use of cryopreserved ovarian tissue to restore ovarian function.
102. If the Minister approves the use of cryopreserved ovarian tissue to restore ovarian function as an established procedure, ACART will continue to monitor international developments associated with the use of cryopreserved ovarian tissue to restore ovarian function.

**Feedback questions (see pages 78 and 79)**

Question 5

- (a) Do you agree that the use of cryopreserved ovarian tissue to restore ovarian function should become an established procedure?
- (b) Do you have any other comments?

Question 6

- (c) Do you agree with ACART's position that the scope for the use of cryopreserved ovarian tissue to restore ovarian function be limited to the woman from whom the tissue was excised, for her own treatment?

Question 7

- (d) Do you have any further comments to share with ACART?



# Glossary

---

<b>Advisory Committee on Assisted Reproductive Technology (ACART)</b>	The advisory committee established under New Zealand's Human Assisted Reproductive Technology Act 2004.
<b>Assisted reproductive procedure</b>	The Human Assisted Reproductive Technology Act 2004 defines an assisted reproductive procedure as a procedure performed for the purpose of assisting human reproduction that involves: <ul style="list-style-type: none"><li>• the creation of an in-vitro human embryo, or</li><li>• the storage, manipulation or use of an in-vitro human gamete or an in-vitro human embryo, or</li><li>• the use of cells derived from an in-vitro human embryo, or</li><li>• the implantation into a human being of human gametes or human embryos.</li></ul>
<b>Cryopreservation</b>	The freezing and storage of tissues and cells at extremely low temperatures.
<b>Established procedure</b>	A procedure that is declared established under section 6 of the Human Assisted Reproductive Technology Act 2004 and therefore does not require approval from ECART.
<b>Ethics Committee on Assisted Reproductive Technology (ECART)</b>	The ethics committee established under New Zealand's Human Assisted Reproductive Technology Act 2004.
<b>Fertility Services Standard</b>	A standard issued under the Health and Disability Services (Safety) Act 2001 that sets out the safety and quality measures that all fertility services provided by New Zealand fertility clinics must meet.
<b>Gamete</b>	An egg or sperm, whether mature or not, or any other cell (whether naturally occurring or artificially formed or modified) that (i) contains only one copy of all or most chromosomes and (ii) is capable of being used for reproductive purposes.
<b>Gonadotoxic</b>	Having a harmful effect on the gamete producing organs ie. the ovary in the female or the testis in the male.
<b>Human Assisted Reproductive Technology Act 2004 (HART Act 2004)</b>	An act to secure the benefits of, and regulate, assisted reproductive technology and human reproductive research.
<b>Informed consent</b>	A person's voluntary agreement, based on adequate knowledge and understanding of relevant information, to participate in research or to undergo a diagnostic, therapeutic or preventive procedure.
<b>In-vitro fertilisation (IVF)</b>	The uniting of egg and sperm outside the body (in the laboratory).
<b>Kaitiakitanga</b>	Guardianship.

<b>Mana</b>	A concept that implies authority, influence and prestige, as well as the recognition of these qualities.
<b>Oocyte</b>	An egg produced by the ovary.
<b>Ovarian function</b>	The ability of the ovary to produce reproductive hormones and potentially release eggs.
<b>Ovary</b>	The egg-producing reproductive organ found in females.
<b>Whakapapa</b>	The genealogical descent of all living things from the gods to the present time.
<b>Whanaungatanga</b>	The obligation of care and support among relatives.

# Appendix 1: Report on the current status of the use of cryopreserved ovarian and testicular tissue for the Advisory Committee on Assisted Reproductive Technology (ACART) of New Zealand

---

Claus Yding Andersen, MSc, DMSc  
Professor of Human Reproductive Physiology  
Laboratory of Reproductive Biology, University Hospital of Copenhagen  
Faculty of Health Science, University of Copenhagen  
Copenhagen, Denmark

Email: yding@rh.dk

Copenhagen December 2014

Note: ACART has previously commissioned a technical report on the use of cryopreserved ovarian tissue that followed the same general structure as below. That report's conclusions were based on evidence up until June 2010. To avoid repetition, we suggest this updated technical report only summarize the work to June 2010 using the previous report as a base, and focus on the developments and clinical outcomes from June 2010 to present.

## Introduction and summary

This report aims to provide a summary of the current knowledge of the use of cryopreserved ovarian and testicular tissue in humans with a vision of informing the Advisory Committee on Assisted Reproduction Technology of New Zealand on the possibility of undertaking these procedures in New Zealand now or in the near future. The present description is a continuation of the report by Professor Richard Anderson, Edinburgh University, Scotland from 2010 and has mainly focused on information which has appeared since this first report.

The brief of this report is that it should be based on published peer reviewed research, be fully referenced and identify areas where there is deficient information. As the clinical application of cryopreserved ovarian tissue is more advanced than the use of testicular tissue the main focus is on ovarian tissue and only to a lesser extent on testicular tissue. For both types of tissue, two main steps are involved in its use, the first being the actual cryopreservation procurement itself and the second part is the treatment of grafting back tissue to the person from where it originated after a storage period. This report attempts to treat these two steps separately.

Since the initial report in 2010 there has been substantial increase in the number of cases undergoing the procedure with storage of both ovarian and testicular tissue. Furthermore the clinical experience in treatment by grafting tissue is ever increasing for ovarian tissue, whereas no well documented clinical cases have yet undergone transplantation with testicular tissue. However, even though the procedure for ovarian tissue cryopreservation is approaching standard practice in some countries, a recent update by the American Society of Clinical Oncology (ASCO) clinical guidelines still categorized cryopreservation of ovarian tissue as an experimental procedure (Loren et al., 2013). However, both ASCO (Lee et al., 2006) and the American Society for Reproductive Medicine Ethics Committee (2005; 2013) acknowledge fertility risks in connection with gonadotoxic therapies and recommend that preserving fertility should be discussed with patients before initiating treatment, especially in light of available data indicating that most cancer survivors prefer to have their own biological children (Lee et al., 2006). In parallel with continuously increasing survival rates of cancer patients and the increasing number of patients it is likely that the procedure will become standard practice in the foreseeable future.

The safety issues of transplanting ovarian tissue to women who at the time of tissue harvest had a malignant disease is now moving from a variety of laboratory tests including grafting to rodents to ascertain safety, to observations in women who actually have had tissue transplanted. Women with grafted ovarian tissue do not appear to experience relapse more often than this group of cancer survivors does without having grafting of ovarian tissue. These observations provide more solid information on the safety and reflect the real clinical situation, where a relatively large amount of tissue is transplanted rather than just a single piece of ovarian cortex normally evaluated in laboratory.

The main indications for excising of gonadal tissue are still malignant diseases or diseases that require gonadotoxic treatment during which the person runs a considerable risk of becoming infertile and has essentially not changed since the first report was released in 2010. However, there is an increasing awareness that the ovaries in addition to provide gametes also undertake an important endocrine function being the main source of sex-steroid hormones in women. Since 2010 two reports showing that ovarian tissue may be used to induce puberty via steroid production in young girls who had a disease in the early years that rendered them sterile (Poirot et al., 2013; Ernst et al., 2013). Use of ovarian tissue not focusing on fertility is now being discussed including postponing menopause in order to avoid osteoporosis, cardiovascular diseases and other menopausal related effects. So, in essence the discussions around fertility preservation and storing gonadal tissue including ovarian tissue are slowly expanding from 1) survival of the patient with only little focus on fertility preservation to 2) quality of life after treatment with avoidance of long-term adverse effects on fertility, to 3) possible use beyond fertility for instance to potential postpone normal menopause. The latter part is not for fertility purposes but utilize the endocrine effects of the ovaries, although these two effects are closely linked.

Therefore, this area is constantly evolving and has not yet found its natural level of activity. The current report attempt to provide a snapshot of the developmental status today but does also outline some of the potential future developments which are now currently not implemented in clinical practice in order to provide ACART of New Zealand with the most up to date information to make potential decisions on.

Anyway, more requests from patients facing gonadotoxic treatment and increasing clinical activities for fertility preservation is seen in number of states worldwide and is most likely to continue in the coming years. To what extent the above mentioned areas focusing on the endocrine effects will become incorporated into clinical practice (and if it is legally allowed) is still an open question. It is important to notice this latter indication has not been used as the only course of cryopreservation of ovarian tissue anywhere in the world at this point in time. However, an estimated more than ten thousands women have already cryostored tissue on a worldwide basis for fertility purposes, which they may or may not need to complete their family and therefore may have available once they reach menopause.

Cryopreservation of testicular tissue for fertility preservation purposes is now being implemented in a number of European states and in the US. Currently an estimated around 300 cases have worldwide had tissue cryostored. Although no well documented human studies on transplantation of frozen/thawed tissue has yet been performed, a number of approaches are now being tested in laboratory animals with promising results also from non-human primates. So this is early days for testicular tissue. However, the risk of the operation against the potential future benefit is now switching towards cryostorage of tissue (McCook, 2013).

## **Definitions used**

*Procedure:* In the context of this report “procedure” is used to define the processing and procurement of the ovarian or testicular tissue mainly comprising the actual cryopreservation process.

*Treatment:* treatment with cryopreserved ovarian or testicular tissue is a wider concept that includes cryopreservation of tissue, storage, noticeably replacement of tissue or cells, monitoring of ovarian or testicular activity and potentially the use of IVF or ART for fertility purposes.

In humans treatment has only been performed with ovarian tissue and not with testicular tissue. It is important to realise that many years may elapse between the cryopreservation of tissue (i.e. the procedure) and then the actual treatment with frozen/thawed tissue (i.e. treatment). In theory, this period may actually last many decades for all we know at the moment.

Consequently, there is a huge discrepancy between the number of young girls and women who have had tissue frozen and those who have had it transplanted. An estimated ten thousands women have had tissue cryopreserved, whereas an estimated 200 women have received transplantation worldwide. In contrast, no man has yet had testicular tissue transplanted – or only poorly documented cases (Radford et al., 1999; Brook et al., 2001; Radford 2003) – but cryopreservation now takes place in several countries.

*Gonadal tissue:* The collective definition of tissue or organs that contain germ cells from either male or female, noticeably a term to collectively describe ovarian and testicular tissue.

*Spermatogonial stem cells (SSC)*: This source of stem cells is the precursors for sperm cells throughout the entire life. As true stem cells they possess the capacity to either self-renewal or through a differentiations process mature into specialised mature sperm cells capable of fertilisation.

Abbreviations:

ESHRE	European Society for Human Reproduction and Embryology
ICSI	Intra Cytoplasmic Sperm Injection
ISFP	International Society of Fertility Preservation

## **A Current status of the procedures in human subjects**

In regard to the use of cryopreserved ovarian tissue and the use of cryopreserved testicular tissue (we acknowledge that the use of cryopreserved testicular tissue may not be at the same stage of the use of cryopreserved ovarian tissue. Therefore, there may be gaps in answering the questions below in relation to testicular tissue).

### **A.1 Indicate if the procedure has been “approved” for human use in other countries. Or alternatively indicate that the procedure has not been banned and is being used for reproductive purposes in other countries.**

*Ovarian tissue cryopreservation*: The specific national rules for conducting this procedure are currently different in almost every country but may overall be performed with a level of approval which can be categorised into one of four levels:

- 1) Approved by the health authorities. This may either lead to a rate or a fee released in connection with the procedure or is paid by the patients themselves.
- 2) Conducted through an ethical approved project. The procedure may either be paid by patients themselves or by the hospital/clinic that perform the procedure.
- 3) The procedure is unregulated and performed at the request of the patient and at the discretion of the clinic. Usually the patient is required to pay for the procedure.
- 4) The procedure is actively banned.

Treatment (i.e. transplantation) using frozen/thawed ovarian tissue for transplantation is less advanced in terms of which conditions apply to its use. No health authorities have yet implemented a rate or a fee for the grafting procedure and cost of the grafting procedure may be free (as in countries with a public health care system) or the patient herself needs to pay the cost. The treatment can be categorised into the following levels:

- 1) Performed at the discretion of the treating doctor under the general rule “do no harm”.
- 2) Conducted through an ethical approved project.
- 3) The procedure is unregulated and performed at request of the patient and at the discretion of the clinic.
- 4) The procedure is actively banned.



*Testicular tissue cryopreservation:* Basically follow the same rules but with the exception that no transplantations – or only very poorly documented cases (Radford et al., 1999; Brook et al., 2001; Radford 2003) – have yet been reported.

## **A.2 Identify possible ‘uses’ of the procedure noting specific situations where this procedure has been used e.g. medical conditions, social reasons etc.**

The use of frozen/thawed ovarian tissue may be considered a way to restore ovarian function. The definition of the ovarian function is for practical purposes twofold:

- 1) To provide fertilizable oocytes.
- 2) To produce sex steroids.

The latter point which in addition of being mandatory for establishment of a pregnancy, also includes numerous endocrine functions, for instance maintain bone mineral density and reduce the risk of cardiovascular diseases. This implies that cryopreservation of ovarian tissue may take place for two fundamentally different purposes:

- a) To possibly restore fertility later on or
- b) To restore menstrual cycles and hormonal activity later on or a combination of both.

The two functions of the ovary are interrelated since fertility requires development of follicles (with an oocyte) that also secure release of ovarian sex hormones including oestrogens and progestin's. Actually the preovulatory follicle secretes more than 90% of all oestradiol present in the second half of the follicular phase of the menstrual cycle and is basically the “sex steroid producing factory” of the ovary. However, the two effects may be separated or combined depending on the physical site in the body in which the tissue is subsequently transplanted. For instance tissue may be transplanted outside of the ovary proper under the skin where it will resume function and release sex steroids to circulation but where a spontaneous pregnancy is excluded.

Currently the ovarian tissue cryopreservation procedure has been used in the following conditions:

- 1) Young girls (i.e. prepubertal) and women with a malignant cancer disease in which appropriate treatment may destroy the ovarian pool of follicles (Rosendahl et al., 2011; Andersen et al., 2013).
- 2) Young girls and women with a genetic disease (for instance Turner Syndrome, Galactosaemia, Thalassemia, BRCA-gene carriers) (Borgstrom et al., 2009; Poirot et al., 2013).
- 3) Patients suffering from for instance disease considered non-malignant such as aplastic anaemia, autoimmune systemic lupus erythromatosis, fragile X-syndrome and endometriosis (Henes et al., 2012; Rosendahl et al., 2011).
- 4) Ovarian tissue may be used to induce puberty (effect of sex steroid secreted from the developing follicles) in young girls who had tissue excised prior to puberty due to a malignant disease (Ernst et al., 2013) or due to a genetic disease (for instance sickle-cell disease) (Poirot et al., 2013).

- 5) A new potential use of cryopreserved ovarian tissue will be to postpone menopause (i.e. effect of sex steroids), for instance in women, who have a family disposition of osteoporosis. If such women have ovarian tissue frozen in their young years it may be replaced once they have reached menopause and resume ovarian activity with circulating levels of sex steroid, which probably is an efficient method to postpone decalcification of the bones. It turns out that up to half of the total ovarian tissue (i.e. one ovary) can be removed without any apparent effect on the time of menopause (1 year earlier than otherwise) and without affecting the fertility negatively (Yasui et al., 2012; Bjelland et al., 2014).

### *Testicular tissue*

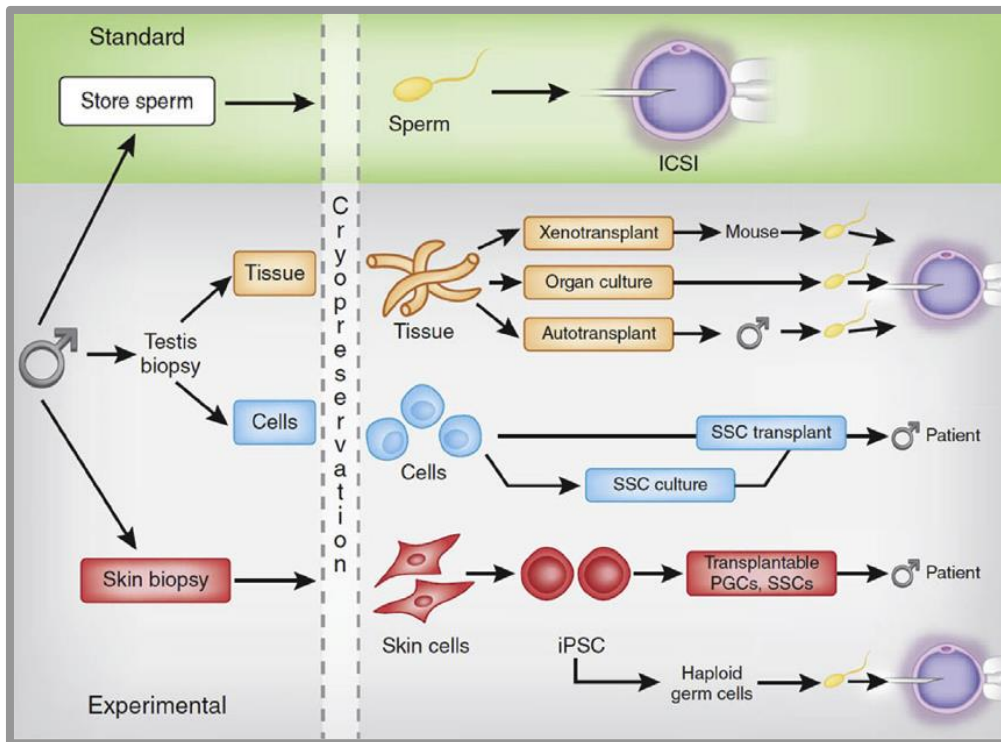
Excision of testicular tissue is currently performed only for fertility preservation purposes, since Leydig cell function and thus androgen (i.e. testosterone) production is less sensitive to gonadotoxic insult (Goossens & Tournaye, 2014; review: Picton et al., 2014). Although it may be envisioned that freezing of testicular tissue can be applied to adult men in the future, only boys unable to produce a sperm sample by masturbation is currently the only group potentially having this procedure offered (review: Picton et al., 2014).

Several different categories of boys have undergone retrieval of testicular tissue:

- 1) Prepubertal boys suffering from a cancer where appropriate treatment poses a risk of destroying all spermatogonial stem cells (Sadri-Ardekani & Atala, 2014; Tournaye et al., 2014).
- 2) Boys with genetic disease (eg. Klinefelter: XXY) that is known to result in infertility with great likelihood in the adult life, but where SSC may still be present in the young years (Rives et al., 2013; Tournaye et al., 2014; Picton et al., 2014).
- 3) Boys with cryptorchidism, often bilateral, where the risk of severe reduced spermatogenesis in adult life. Often there will still be SSC present in the young years, where the testis may be placed in the scrotum during an operation (Kvist et al., 2005).

A specific method for utilisation of the frozen/thawed tissue for production of sperm cells and procreation has only reached the first preliminary stages and no specific strategy has been chosen. In fact a number of alternatives are being persuaded. If more than one of these methods will reach a clinical stage they may be used depending on the diagnosis in the first place and the amount of tissue available.

The different options currently being researched in an attempt to develop clinical procedures is nicely summarised in the figure 1 (review: Valli et al., 2014) and detailed with appropriate references to the original research describing this approach.



**Fig.1. The potential clinical approaches for utilising testicular tissue for fertility preservation (Valli et al., 2014)**

The potential therapeutic options include: A testis biopsy may after a cryopreservation period be transplanted to an immunodeficient mouse that potentially may provide a suitable environment for the development of sperm cells or sperm like cells that have halved their chromosome number and may be used in connection with ICSI.

Alternatively the tissue may be cultured and sperm cells or sperm like cells that have halved their chromosome number developed during culture may be used in connection with ICSI.

Another method and perhaps the method with the least ethical concern and concern over potential side effects are directly grafting the tissue to the patient and preferentially into the remaining testis with the aim of having sperm production ensuring. The sperm may need to be retrieved for use in connection with ICSI.

One more approach is to isolate SSC from the testis biopsy. The SSC may then either be propagated in the laboratory to reach a number sufficient for transplantation or directly be injected into the remaining testis. In both cases the SSC will need to reach the testis tubules and in an appropriate environment initiate spermatogenesis.

The last method for restoring fertility is rather futuristic in connection with a clinical application at this moment. It focuses on potentially to obtain patient specific haploid germ cells and indeed also functional sperm cells through the derivation of induced pluripotent stem cells from for instance a skin biopsy. This elaborate process will include dedifferentiation and erase of imprinted genes in the skin cells and turning these cells into cells resembling human embryonic stem cells. Human embryonic stem cells are present in the five-six day old human embryo, where only very limited specialisation has taken place. The induced pluripotent stem cells will need to be re-differentiated into a germ line in the laboratory, or be directly injected into the patients. Both approaches could potentially result in generation of haploid cells and a clinical approach.

### **A.3 If the procedure has been approved (or is in use), specify:**

#### *A.3.1: which countries*

In October 2013 the EU-patient mobility directive was approved (directive 2011/24/EU) which is explained in an accompanying document from the UK department of Health (UK – Department of Health). This implies that patients who cannot receive a treatment in his or her home county can go to another EU member state to have the procedure or treatment performed and is furthermore entitled to have the cost reimbursed by the health authorities of the home country. The immediate effect of this directive is that a number of European countries are now actively starting freezing programs (for instance Italy where the national transplantation unit now is coordinating a national initiative) and over time this directive is likely to cause member states to adopt similar or almost similar rules for many of these procedures.

As defined in A.1. the procedure is approved by the health authorities (i.e. 1) in: Denmark, Norway, UK, and Israel.

As defined in A.1. the procedure is approved under an ethical licence and is used in: Germany, Switzerland, Sweden, Finland, Holland, Belgium, France, Spain, Italy, UK, USA, Austria, Australia, Japan, Portugal, Poland, Hungary, Singapore.

A number of these countries have performed only a limited number of procedures and are only now getting programs established. The procedure and treatment is covered by the EU-tissue directive, which implies that all EU member states follow the same basic rules (as for instance for testing for disease markers including Hepatitis, HIV, Syphilis) and for maintaining information of equipment and utensils used etc.

As defined in A.1. the procedure is performed (possibly) without regulation in: South Africa, Vietnam, Russia, China, India.

As defined in A.1. the procedure is not currently known to be banned in any country except for Bulgaria where a moratorium on the cryopreservation of gonadal tissue have been implemented until formal regulations will be approved by the authorities.

A number of countries are now starting programs including Jordan covering the Middle East, Vietnam, Hong Kong and many places in China.

## Testicular tissue

According to a survey performed by the ESHRE task force for fertility preservation and recently published six European countries were currently offering cryopreservation of testicular tissue for fertility preservation, including Belgium (two centres), The Netherlands, Sweden, Germany, France and Denmark (Picton et al., 2014).

The interest in starting cryopreservation of testicular tissue in the US has recently been described in Nature Medicine (McCook, 2012; Sadri-Ardekani & Atala, 2014; Tournaye et al., 2014).

### *A.3.2: when approval was given/use began*

Approval of cryopreservation of ovarian and testicular tissue was in Denmark given by the Minister of Health in year 1999 provided that it was only used in the person from whom it originated (i.e. autologous). Use in a different person (i.e. allogenic) was by law forbidden.

In Sweden cryopreservation of ovarian and testicular tissue received started in year 2003 following ethical approval of the procedure and treatment. The procedure was approved and has been performed since the late 1990'ties in Belgium, Israel, France, UK, and Sweden while Germany, Norway and many other countries have only recently started their services.

### *A.3.3: the extent or conditions of the approval/use*

In most European countries the approval allows only use of gonadal tissue by the person from whom it originated (i.e. autologous). Thus, it is illegal to treat another person with the tissue (allogenic).

In most countries the tissue needs to be destroyed in case the person dies. However, it may be used for research purposes if the person donated the tissue when living, but it cannot be transplanted to another person. Transplantation to another person is also difficult anyway because of tissue rejection reactions similar to those that will occur if any tissue at random is transplanted to another person.

In Norway the tissue cannot be harvested from for instance women with cervical cancer, since this person will subsequent need a surrogate mother to conceive and that is illegal in Norway. This has the effect that Norwegian women now travel to Denmark to have the tissue frozen. In Denmark surrogacy is also illegal, but it is accepted to graft the tissue to the woman, who will then need go abroad to have IVF and surrogacy performed on her own behalf.

In Denmark it is only legal to perform the procedure in women of iatrogenic induced ovarian failure plus genetic diseases that will lead to premature ovarian insufficiency.

In Scandinavia and many countries in Europe, the procedure is not performed for social reasons, whereas this is allowed other countries like the UK and USA and is linked to the fact that the person covers the cost herself.

#### **A.4 If the procedure has been banned (or has proven to be controversial), specify:**

##### *A.4.1 which countries and A.4.2 why it was banned or proved to be controversial*

The uptake of this new technology has occurred very different round the world. The following examples illustrate some of the concerns that has had an impact on the uptake and use of this method.

- 1) UK: After an initial considerable activity the use of this new technology has been very slow to be implemented up until now. This is mainly due to the fact that leading clinicians were of the opinion that only a minority of the treated women would need the procedure (i.e. most of the women would not enter menopause as a consequence of the gonadotoxic treatment she was facing) and that clinicians claimed to be capable of identifying the women who actually needed it (Wallace et al., 2014).

In addition, the UK approval system is very laborious and costly requiring a lot of work and implementation of many new procedures, which is new to most fertility clinics and requires a lot of effort and attention.

- 2) Apart from a few indications, as for instance bone marrow transplantation (BMT) and heavy exposure to alkylating agents, which almost invariably causes extinct ovarian function, the outcome in terms of gonadotoxicity is difficult to predict on beforehand, especially in children and only limited up-to-date information on the risk of extinct ovarian function is available. One reason is that many treatment regimens are not static, but change over time and may be individualised as the treatment progresses. Further, some treatment regimens used for instance in breast cancer is dependent on the response from the woman during the course of treatment. So if the woman only shows a modest response during the first few rounds of treatment the dose of chemotherapy may be increased (Bergh et al., 2000; Rosendahl et al., 2009).
- 3) Further, every estimate on the risk of gonadal failure, even given a huge risk for any given cancer treatment, will be perceived different by almost every girl/woman. Some may find a risk of for instance 15 percent to be high enough to go ahead with fertility preservation, whereas others will find such a risk too small to undergo the procedure. One aspect as described above is the immediate gonadotoxicity, which occurs in connection with the cancer treatment and shortly thereafter. Another aspect is the long term perspectives, will the girl/woman enter menopause earlier than normal. Most cancer treatments reduce the pool of ovarian follicles to some degree. Currently it is very difficult to estimate the specific effect of a treatment and although the ovaries will maintain menstrual cycles even after having at least half of the ovarian pool of follicles removed (Yasui et al. 2012), it is currently unknown how big a gonadotoxic insult will can be tolerated for maintaining menstrual cycles until normal menopause.
- 4) Finland: The use of this technology in Finland is currently very low and almost non-existing (from the beginning of this century and until today less than one hundred patients have undergone the procedure). The main reason for this reluctant use is that leading clinicians including paediatricians fear that replacement of the tissue will cause reintroduction of the original disease and make the patient sick again. The risk of introducing the original disease will be discussed below. Further many clinicians in Finland are of the opinion that not a lot of these girls and young women will need the procedure.

- 5) USA: In the US this procedure is only performed at the wish of the patient. She is required to cover the cost of the procedure (although some clinics perform the procedure without charging patients). Legally this puts US clinics in a special situation – if the tissue subsequent is grafted to a patient that previously suffered from a cancer disease and that patient experience a relapse (whether it is caused by the grafting or would have happened anyway) a law-suit may be claimed. Did the replaced tissue cause introduction of the disease? At this point in time it is impossible to exclude that this is the case with 100 percent certainty irrespective of the fact that statistically a fraction of patients recovered from most cancer diseases will experience relapse. Therefore by performing treatment with this procedure the clinic runs the risk of being judged to pay substantial reimbursement if the patient have relapse.

Currently leading clinicians work on having ovarian tissue cryopreservation acknowledged as non-experimental, which is the critical step in having insurance companies to cover fertility preservation.

- 6) Collectively, the judgement from many clinicians is that the procedure and treatment is still in its infancy and that they will wait until more solid proof of the benefits is accumulated.

#### **A.5 Indicate the number of individuals who have used the procedure and/or the number of individuals studied who have used the procedure**

There is no collective account of the number of girls and young women who have had ovarian tissue cryopreserved. The ISFP and ESHRE have launched initiatives to collect information from around the world. However, there are no results published from these initiatives yet. Usually the figures on the number cases having had ovarian tissue cryopreserved itself do not warrant publication, but some information from peer-reviewed papers is available.

- Germany and the German speaking countries Austria and Switzerland in the Fertiprotekt network perform by far the largest activity in this field worldwide. At their website it is stated that the number of women having ovarian tissue cryopreserved is: Year 2007:130; 2008:190; 2009:250; 2010:230; 2011: 255; 2012:305; 2013:390. In total this amounts to 1750 cases. These data are on their way to be published in a scientific journal but is available at their website ([www.fertiprotekt.de/](http://www.fertiprotekt.de/)). Further it appears that the increase in number continue this year.
- Belgium: The activity of cryopreservation of ovarian tissue from April 1997 and January 2012 by Donnez's group was 476 (Dolmans et al., 2013).
- France: A total of around 2000 patients have had tissue cryopreserved since year 2000 (N. Rives, Rouen, France personal communication).
- Spain: In Valancia a total of 717 patients have had tissue frozen (A. Pellicer, personal communication). Several other centres are performing ovarian tissue cryopreservation.
- Denmark: The activity of cryopreservation of ovarian tissue from 1999 and until November 2009 was reported to be 405 cases (Rosendahl et al., 2011). In November 2014 this figure had risen to 742 cases with a constant number of cases during the last five years of around 13-14 cryopreservation's per million inhabitants per year. Of the Danish cohort 152 were below the age of 18 years.

- Norway: As of year 2007 a total of 22 cases had undergone cryopreservation in Norway (Storeng et al., 2007). This number has substantially increased by now.
- Israel: Between the years 1996 and 2013 around 400 patients have had tissue cryopreserved in one centre (Shapira et al., 2014), while more centres are also performing the procedure.

Children: A total of 266 cases in children below the age of 18 years have recently been reviewed (Donnez et al., 2013) not including figures from Denmark. Among these cases at least 74 were under the age of 10 years.

Collectively it is estimated that around 10,000 women have had tissue cryostored worldwide until 2014.

### Testicular tissue

There is no collective database information. A recent study from ESHRE task force of fertility preservation reported that seven European centres had already started to collect testicular tissue from young boys for fertility preservation (Picton et al., 2014). A total 266 biopsies were cryopreserved from boys aged 0.8–16 years. The cost for the procedure was from a research grant in all except from one centre. Four centres reported that legal regulations applied to the collection, while three centres reported that no regulations applied (Picton et al., 2014).

In Rouen, France the number of cases is reported to be 88 as of December 2014 (N. Rives, personal communication). In Denmark the total number of cases is 22 as of December 2014.

In the US, a number of 16 cases have been reported (Ginsberg et al., 2010; Ginsberg 2011).

In the US, it has been estimated that around 5,100 boys under the age of 15 will develop cancer each year with a survival rate of 83% (review: Valli et al., 2014). The Childhood Cancer Survivor Study indicates 44% of these boys subsequently will be unable to father children resulting in almost 1,900 boys annually who will become sterile (Valli et al., 2014). Further it is interesting that more than 80% of parents were interested in fertility preservation procedures on behalf of their boys (Wyns et al., 2011; Ginsberg 2011).

### **A.6 Describe the information that is available on the outcomes of using the procedure.**

Treatment has only been performed with ovarian tissue in humans and not with testicular tissue. There are basically two outcomes of treatment:

- 1) regaining menstrual cycles (avoidance of menopausal symptoms).
- 2) children born for those who have fertility wish.

*Children born from grafting frozen/thawed ovarian tissue:* The most recent accounts on the number of children born from having frozen/thawed ovarian tissue transplanted published in peer-reviewed paper is 35 (Macklon et al., 2014; Donnez et al., 2014) representing a total of eight countries. All children have been reported healthy. Additionally the health outcomes



for a number of children born as a result of transplanted frozen/thawed ovarian tissue are in the process of being published or are reported as on-going pregnancies (Shapira et al., 2014). It is worthwhile to mention that fertility have been obtained following transplantation in two different situations, 1) orthotopic transplanted tissue including the remaining postmenopausal ovary and the peritoneal cavity or 2) a from tissue transplanted to sites outside of the ovaries (this may be called heterotopic transplantation, but no firm definitions of this term exists).

*Children born from grafting fresh ovarian tissue:* This group of patients mainly represents genetic identical twins where one sister has ceased to have ovarian function and receives tissue from her sister. Since they are genetically identical to their sister they may, without any risk of tissue rejection, obtain tissue from the sister and become fertile. This approach has been used in eleven US twin sisters with a remarkable good result. Having fresh unfrozen tissue transplanted one from sister to the other 11 babies have been reported (Silber 2012) plus one from (Donnez et al., 2012), in total 12.

#### *Regaining menstrual cycles*

Most of the grafted women regain menstrual cycles. A recent study reported a figure of 93 per cent (52 out of 56 women) (Donnez et al., 2013). Another study from Denmark reported that 18 women transplanted with frozen/thawed ovarian tissue all had regained ovarian function for nine to seven years – still ongoing (Greve et al. 2012). The duration of ovarian activity is dependent on a number of parameters the most important being age at excision, the amount of tissue transplanted and efficacy of freezing and grafting. However, the pool of follicles that do become active after grafting is low as shown by constant low levels of AMH (Janse et al., 2011; Greve et al., 2012), but is apparently capable of providing one preovulatory follicle for extended periods of time.

#### **A.7 Describe the information that is available on the risks of using the procedure.**

Recently several detailed reviews have addressed the risk of using this procedure for grafting ovarian tissue excised at the time when the patient experienced active disease (Rosendahl et al., 2013, Bastings et al., 2013a & b; Dolmans et al., 2013). These reviews nicely present the available data on residual disease in ovarian tissue prepared for transplantation. However, the actual clinical situation is difficult to mimic in an ex vivo situation and only in case of a positive answer for contamination may the result prove valid. There are a number of issues that needs to be considered and which have only limited information on today as discussed below.

- 1) Although women who died from cancer showed metastasises to the ovaries in a relatively large fraction (Kyono et al., 2010) they all suffered from end-stage disseminated disease and earlier stages of disease in a number of cancers do not appear to readily spread to the ovaries. Today many patients are only offered cryopreservation of ovarian tissue provided that the disease is not disseminated and therefore often in earlier stages. However, this raises the question: At what disease stage does ovarian involvement begins? For most cancers this question unanswered.

- 2) The question is how many cells grafted are necessary to cause relapse, in other words what is the Minimal Infectious Dose (MID) (Yding Andersen, 2014). It is well known that some cancer patients maintain to have malignant cells present in low number in circulation after being disease free as for instance chronic myeloma leukemia (CML)(Ito, 2013). Will the presence of a few cancer cells in the ovarian tissue in this situation actually cause relapse? This information is unknown for CML and most other cancers, but studies in rats have shown that just a few malignant leukemic cells were sufficient to cause relapse (Hou et al., 2007; Jahnuainen et al., 2001). However, old studies in American prison inmates showed that injection of even very large amounts of malignant cells from one person to the other did not introduce disease (Lanman et al., 1950). So, apparently the immune system is capable of handling even large amounts of disease cells. Indeed, many of the patients suffering from for instance leukemia actually undergo bone marrow transplantation (BMT) and receive an immune system from another person. It is unknown whether this new immune system may handle a few contaminating cells in connection with grafting ovarian tissue?
- 3) The number of malignant cells transplanted is dependent on the number of pieces of ovarian grafts. This may vary from just a few to maybe 12–16 and will impact on the number of malignant cells being potentially transplanted. However, the MID in connection with a BMT and transplantation of ovarian tissue is completely unknown. So, for each individual patient it is probably unlikely that any test will provide one hundred percent accuracy on detecting the risk of relapse in connection with grafting ovarian tissue collected during active disease. Except for actual transplantation results involving patients, the below mentioned data will only in case of a confirmatory result for malignant cell contamination provide useful information on the risk of the procedure.

Collectively, safety of grafting of ovarian tissue in this point of time is still unresolved. Reassuring, however, is the actual clinical data as presented below, which seems to support that malignancy is not grafted by the current policy for cryopreservation of tissue.

To complete the answer to this question it should be added that there also potentially could be a risk of having the procedure (procurement of the tissue) performed including the risk of undergoing the operation for removal of ovarian tissue.

#### *Animal and primate models*

Using immunodeficient mice as a bio-incubator allowing cell proliferation and grafting ovarian tissue from patients with suspected malignant cell contamination is currently considered the most sensitive method for risk evaluation. However, immunodeficient mice are expensive, the method is time consuming and requires an expertise that many fertility clinics and transplantation centres may not have available. Further the so called take-rate, the frequency of development of malignancy following deliberately transplantation of malignant cells, is usually not 100 percent and may be considerably lower and dependant on the number of malignant cells being transplanted (Greve et al., 2013).

*Leukaemia* Leukaemia usually includes diseases such as acute lymphocytic (ALL), chronic lymphocytic (CLL), acute myeloid (AML) and chronic myeloid (CML). Leukaemia is usually considered the disease with the highest risk of causing relapse in connection with grafting,

because leukaemia is a disease of the blood and malignant cells will almost invariably be present in the tissue. Ovarian tissue from patients who suffered from leukaemia at the time of tissue harvest has been evaluated in immunodeficient mice in two recent studies. In the first study by Dolmans and co-workers (Dolmans et al., 2010) five out of 18 immunodeficient mice transplanted with ovarian tissue developed human leukaemia. The results clearly indicated that it was not advisable to transplant tissue to these patients confirming an earlier study using only *in vitro* tests (Rosendahl et al., 2010). In another recent study 24 patients who all suffered from different types of leukaemia had frozen/thawed ovarian tissue transplanted to immunodeficient mice for 20 weeks (Greve et al., 2012). In contrast to the first study no viable malignant cell contamination could be detected in any of the 24 patients. However, it turned out that the majority of patients in the latter study were in complete remission at the time of tissue collection. Complete remission implies that no malignant cells is seen in circulation but potentially may be present in the bone marrow (Greve et al., 2012). One or a few series of chemotherapy is normally applied to accomplish complete remission, which, however, did not affect the density of primordial follicles in the ovarian cortex as compared to other age matched patients that had not experienced prior gonadotoxic treatment (Greve et al., 2012). Therefore it is now recommended to collect ovarian tissue from leukemic patients when they are in complete remission (Dolmans et al., 2013) although collection of more studies with a higher number of patients is necessary for grafting to become a wider application to this group of patients.

Further a recent study evaluated ovarian tissue from one CML patient with sensitive molecular biological methods (Abir et al., 2014). The study found traces of malignant cells in ovarian tissue, which in the case of this patient resulted in grafting stopped. However, the same group has reported transplantation of ovarian tissue to a former CML patient.

The most comprehensive analysis of presence of sarcoma cells in ovarian tissue prepared for transplantation currently performed included a total of 16 patients of which nine had Ewing sarcoma and the remaining other types of sarcoma (Greve et al., 2013). Ovarian tissue was transplanted to immunodeficient mice for 20 weeks and evaluation for the presence of the Ewing sarcoma specific *EWS/FL1* translocation in all cases revealed no signs of malignant cell contamination (Greve et al., 2013). However, another study found one case of ovarian tissue positive for the *EWS/FL1* translocation out of eight evaluated patients (Abir et al., 2010). Recently another study reported on the presence of CD99 positive cells on the surface of ovarian tissue harvested from a Ewing sarcoma patient (Sørensen et al., 2014). Apparently only few studies have specifically looked for ovarian involvement in early stage Ewing sarcoma (Rosendahl et al., 2013). Recently, the presence of the *EWS/FL1* translocation was detected with a highly sensitive Q-PCR technique. No signs of malignant cells were present in any one piece of ovarian cortex representing eighty percent of one entire ovary (Yding Andersen et al., 2014). Collectively, the data suggests that Ewing sarcoma and other types of sarcoma do not readily localize to the ovaries, but it cannot be excluded with a 100% certainty (Greve et al., 2013).

A number of other diseases including breast cancer, lymphoma and cervical cancer have been evaluated for possible malignant cell contamination and have been described in recent reviews (Rosendahl et al., 2013, Bastings et al., 2013a & b; Dolmans et al., 2013). The overall conclusion today is that malignant cells contamination of ovarian tissue collected from any one type of cancer cannot be excluded, the risk increases with advancement of the

cancer stage and disease in any given woman, but the generally the risk is very low. However, these studies are gaining less interest in parallel with the number of women who actually experience grafting is rapidly increasing.

*Actual transplantation to girls/women*

*Reported relapses of cancer disease in women transplanted with frozen/thawed ovarian tissue*

A total of six cases in which the woman experienced a relapse after having had frozen/thawed ovarian tissue transplanted have currently been reported. This information should be view with the background that an estimated around 200 cases have been transplanted worldwide with approximately 120–150 cases in which the woman had a cancer disease at the time of tissue retrieval.

<b>Diagnose</b>	<b>No. women</b>	<b>Reference</b>
Mammae cancer	2	Schmidt et al., 2010; Ernst et al., 2013
Cervical cancer	2	Kim et al., 2009
Granulosa cell tumor	1	Stern et al., 2014
Ewing sarcoma	1	Yding Andersen et al., 2014

The tumor relapses occurred at the original site of the tumor and there has been no indication of the ovarian tissue causing the relapse. One exception may be the granulosa cell tumor where the relapse may actually have occurred from the grafted tissue.

The case with the Ewing sarcoma patient is illustrative (Yding Andersen et al., 2014): The patient had one of her ovaries frozen at nine years of age prior to undergoing treatment for Ewing sarcoma. When she was 13 years and had no ovarian activity, two of ten pieces of ovarian cortex were grafted to the remaining postmenopausal ovary. The grafted tissue regained activity and stimulated her pubertal development. In 2013 four and half year after the tissue was grafted, then aged 18 years, the patient experienced a relapse of the original disease with Ewing sarcoma occupying the whole left thoracic cage and metastases in the liver. During the subsequent 7 months, the patient received intensive treatment but died in January 2014. Although the relapse occurred in the hemithorax away from the site of the remaining ovary, the remaining eight pieces of ovarian cortex prepared for transplantation were tested for the possible presence of malignant cells. The patient was positive for the EWS/FL1 translocation. The eight pieces of ovarian tissue were thawed and each piece was divided into six smaller fragments of tissue. Each individual piece was subjected to a manual homogenization and extraction of mRNA but the EWS/FL1 translocation was not detected in any sample. These data indicate that the ovarian tissue did not contain malignant cells. However, since the two originally transplanted ovarian pieces of cortex were not analyzed for the translocation, it cannot with 100 percent certainty be excluded.

Collectively, at present the relatively large number of actual transplantations to girls and women suggests that no major health risk is associated with the treatment of the tissue. The treatment is far from being fully evaluated and further studies are required to

substantiate the safety, especially in connection with leukemia and tumors originating in the ovary itself as for instance granulosa cell tumors. However, it is envisioned that the development from now on to a large extent will be clinically driven.

**A.8 Describe the information that is available on the benefits of using the procedure, including whether there are potential recipients of the technology who would otherwise have no available option.**

Those girls and young women who currently undergo the procedure are at risk of losing the ovarian pool of follicles, in which case they become sterile. If sterility ensues they are likely to benefit from the procedure. A Danish study interviewed women who had undergone the procedure (i.e. removal of one ovary) and 80% of the women stated they would use the tissue if it becomes necessary (Schmidt et al., 2013). The other side of the coin and perhaps equally important, did the procedure caused harm? Avoidance of harm is one the medical profession founding pillars. A Danish study showed that women who did not enter menopause as a result of cancer treatment, and who had one ovary frozen prior to treatment (and therefore potentially did not need the procedure), did not experience a reduction in fertility and 72 present of those women had actually already at the time of the study achieved the children they wanted (Schmidt et al., 2013). This is important information because it is currently very difficult to predict who will and will not become infertile. There will be women who do not experience menopause immediately in connection with the gonadotoxic treatment. Further, the overall goal of the procedure is to maintain ovarian function as close to the normal situation as possible for (childhood) cancer survivors, which also should involve an aim of allowing entry into menopause at the same age as normal women. There does not exist follow-up data for long enough to know whether these women actually enter menopause prematurely and potentially could benefit from their tissue.

The patients who as a result of the grafted of tissue got a child obviously benefitted from the procedure. In addition to that the following information is available:

- 1) Many women find it very stressful to undergo cancer treatment and obviously a life-threatening diagnosis is severe. Evidently all patients want to survive, but immediately after that many patients express great concern on their future fertility (Treves et al., 2011; Treves et al., 2014). Therefore fertility preservation including cryopreservation of ovarian tissue is often a great relief for patients and physiologically important for them going through often harsh treatment. The following quotes from patients illustrate this point: 1) "Prior to exposure to chemotherapy and radiology therapy I wish to save my genetic material in order to use the same when restored to health. This is my one and only treasure and presently the main, if not the only, reason for fighting this illness"; 2) "the fact that the doctors advised me to have ovarian tissue cryopreserved made me believe that they actually thought I would survive the disease"; 3) "preserving my fertility was the light at the end of the tunnel for me going through chemotherapy" (Schmidt et al., 2013).
- 2) Young prepubertal girls with a malignant cancer or genetic disease in which appropriate treatment may destroy the ovarian pool of follicles have no other option for preserving fertility. In two cases the tissue has been used to induce puberty (Poirot et al., 2013; Ernst et al., 2013). These girls may in their adulthood return for subsequent tissue to become fertile. The time frame in which freezing have been

performed is still too short for prepubertal girls who had tissue frozen to return to use it for fertility, but the first cases are likely to come back in the near future.

- 3) In Denmark seven women have until now had tissue grafted not to regain fertility but “merely” to become normal young women again with menstrual cycles and avoid menopausal symptoms. However, they do have the option of using the grafted tissue for fertility purposes if they should wish so.

#### **A.9 Describe any areas where there is deficient information about the procedure (i.e. potential risks, benefits and outcomes).**

Long term follow-up studies focussing on the children and their development are lacking. In additions, studies is missing following the grafted women in order to secure that the transplanted tissue does not develop into malignancy later on. Solid answers to these questions are estimated to be at least ten years in the future.

Potential negative consequences for girls and women having tissue removed including immediate surgical complications or potential reduced fertility later in case they do not become menopausal from the treatment they are facing also needs further studies although there is already some information, which suggests that this is not major issues (see above).

The success rate of the transplanted tissue in terms of children born is also uncertain and will only be accessible once a large cohort of women who have had frozen/thawed tissue transplanted for fertility purposes experience exhausted ovarian function and do not have more tissue stored in the freezer. Only at this point in time can the nominator and denominator in the equation of success be defined and evaluated (see section 10.3). This is estimated to last another five to ten years before this information will be available, but preliminary data are now available from Denmark (see below).

Women who have had tissue frozen, whether they experience premature ovarian insufficiency or will enter menopause at a normal age, will have the option of having benefits from the tissue when they have entered menopause as a possible source of sex steroids to avoid menopausal symptoms (not to become fertile and become “old mothers”). In the future the health care implications of the longer life expectancy are tremendous (the ageing population) and the cost to society is huge and will increase (Riggs and Melton, 1995). Although the main goal of ovarian cryopreservation is fertility preservation, transplantation of frozen-thawed ovarian tissue may serve as a physiological and natural solution to prevent the massive medical legacy of osteoporosis in our ageing population (Yding Andersen & Kristensen, 2015). In this scenario, the general population may want to use the method and this technique may have consequences far beyond fertility preservation This obviously does not exclude women who have had tissue cryopreserved for fertility preservation for instance women with cancer from having the benefit of both possibilities (see point 5 in section A2).

## **B. Information from human studies (review if applicable)**

### **B.10 Outline the efficacy of using cryopreserved ovarian and testicular tissue, including:**

#### *B.10.1 fertilization rates*

Women who undergo IVF treatment following transplantation of frozen/thawed ovarian tissue show a lower fertilization rate of the retrieved oocytes than other women (Dolmans et al., 2009; Schmidt et al., 2010).

#### *B.10.2 survival rate of the retrieved oocytes following cryopreservation and then re-implantation*

Only one study in Spain has reported on the possibility of freezing oocytes after having replaced frozen/thawed ovarian tissue (i.e. a second cryopreservation). This study resulted in the delivery of twins after four cycles of IVF in one patient in which a total 18 oocytes were retrieved. Nine of these oocytes were vitrified, two of which later gave rise to the twin gestation (Sanchez-Serrano et al., 2010). This result basically just illustrate that it is possible to perform freezing twice and still have available viable oocytes and does not provide information on this method as a more widespread approach.

#### *B.10.3 pregnancy rates (please compare to use of fresh mature oocytes in IVF) and B.10.4 live birth rates (please compare to use of fresh mature oocytes in IVF)*

Please have a look at section A.9.

It is difficult and probably doesn't make sense to make a direct comparison to IVF results. In IVF treatment a definite answer is available after a short period (i.e. two weeks) after having transferred the embryo. For ovarian tissue transplantation a pregnancy may occur as long as the tissue remains active and the tissue has in some case been shown to be active for more than seven years (Yding Andersen et al., 2013). In Denmark two women conceived five years after having tissue grafted and obviously the pregnancy rate will increase as long as the tissue remains active (Macklon et al., 2014).

Furthermore, it is difficult to obtain reliable data on the pregnancy potential of transplanted frozen/thawed ovarian tissue since there currently is no international data base where clinics report their results. As the method becomes more and more widespread with more centres having pregnancies it becomes increasing difficult to publish data on small series or indeed case reports on pregnancies in peer-reviewed journals.

Just recently, this was illustrated in an article published in The Lancet, where data on the pregnancy rates in women having ovarian tissue transplanted turned out to be misleading (Scoop et al., 2014), mainly because data were not based on published results. Subsequent the data were corrected and more accurate data for four clinics including the Danish programme was published (Donnez et al., 2014). In this latter publication a figure of 23% was reported, which, however, already in the Danish program has been increased (unpublished data).

### *B.10.5 diagnostic accuracy of the procedure*

The diagnostic accuracy of predicting the group of women who is most likely to become pregnant following transplantation of frozen/thawed ovarian tissue no reliable data are currently available. However, in a series of patients it appears that the younger the woman was at cryopreservation the more likely she is to become pregnant subsequent to transplantation (Donnez et al., 2013), which is a result similar to that observed for IVF patients and the general population as such.

## **B.11 Detail any risks to health through the use of cryopreserved ovarian and testicular tissue, including (but not limited to):**

### *B.11.1 any potential side effects (please compare to use of fresh mature oocytes in IVF)*

There have been no reports on any potential risks to health through the use of cryopreserved ovarian tissue in terms of oocytes and subsequent embryos being more abnormal or less viable as compared to mature oocytes obtained in IVF treatment. A relatively large proportion of women (up to around 50%) of the women transplanted with ovarian tissue conceive spontaneously and are not exposed to any greater risk of side effects as normal women who become pregnant (Macklon et al., 2014). It is almost exclusively the resting primordial follicles that survive freezing and transplantation. These follicles must undergo a four to six months growth and development phase to reach the preovulatory stage where fully mature oocytes are available and released for fertilization. Suboptimal follicles are likely to become atretic and disappear during this process, exactly as it occurs in the natural development. Therefore the preovulatory follicles will most likely be similar to those that develop naturally.

### *B.11.2 health outcomes for female patients. This would include both short term and long term consequences of the procedure (e.g. the treatment could increase the risk of cancer many years later)*

Excluding the potential risk of malignant cells from the original cancer disease being present in the transplanted tissue, there are no sign and information available to suggest that the transplanted tissue should increase the risk of cancer later on. In Denmark the first transplantation was performed in year 2003 (Schmidt et al., 2004) and since then more than 30 women have had tissue transplanted with 15 women having had tissue transplanted for more than five years. So far none of these women have developed any types of cancer as a result of the grafting, but obviously the information is still very limited and long term follow-up studies are required to obtain more definite answers to this question.

### *B.11.3 any suggested exclusions of potential patients based on clinical indicators (e.g. cancer, diabetes)*

Possible exclusions of patients that may qualify for transplantation of ovarian tissue should be no different from other women, where a pregnancy may carry a risk to the woman herself; this could be the case for diabetes or Turner syndrome (oocyte donation).

Women who had a cervical cancer may be without a uterus or have a damaged uterus that is unlikely to be able to carry a pregnancy to term and they may require a surrogacy mother



in order to get their own children. The local legislation may therefore impact on the suitability of doing the cryopreservation in the first place.

Further, in case of patients who had an oestrogen sensitive breast cancer, it could be hypothesised that high levels of oestrogens as observed during a subsequent pregnancy could increase the risk of experiencing relapse. However, relative good evidence suggests that these patients once disease free has no increased risk of becoming pregnant (von Schoultz et al., 1995; Kroman et al., 1997).

A special case is cancers of the ovary itself. Replacing frozen/thawed ovarian tissue will invariably introduce the original source of malignancy. Since the ovarian tissue already once has developed malignancy (most likely the contralateral ovary) there is probably an increased risk that this will happen again. The woman would not be exposed to this risk unless the tissue is transplanted. One such case has recently been reported from Australia (Stern et al., 2014) in which a patient who previously suffered from a granulosa cell tumour had ovarian tissue transplanted twice subsequent to becoming disease free. She became pregnant and gave birth to a twin delivery but the cancer reappeared during the pregnancy. Following childbirth the patient had undergone renewed cancer therapy but is now reported well again (Stern et al., 2014). It is uncertain if the cancer was actually present prior to transplantation of ovarian tissue, but there is a risk that it was introduced in connection with transplantation.

#### *B.11.4 observed damage to the oocytes*

Women transplanted with frozen/thawed ovarian tissue will have a low ovarian reserved witnessed by low circulating AMH levels (Greve et al., 2012). The collective experience of performing controlled ovarian stimulation and subsequent IVF treatment of these patients have shown a poorer oocyte retrieval rate and a poorer embryo development rate as compared to IVF patients undergoing the procedure with a different indication. This may imply that the oocytes and follicles have been damaged during the cryopreservation procedure. However, another reason may be that the much reduced selection of follicles during growth and development leads to development of follicles with a reduced developmental potential and which during normal circumstances would have been de-selected (Greve et al., 2012).

#### *B.11.5 any issues related to maturation of sperm from cryopreserved testicular tissue*

Cryopreserved testicular tissue can serve as the starting point for isolation of SSC. Depending on the size of the testicular biopsy propagation of SSC may be necessary prior to transplantation. However, animal studies have now shown that SSC transplantation is successful in mice, rats, pigs, goats, bulls, sheep, dogs, and monkeys resulting in renewed spermatogenesis and production of donor-derived progeny (review: Valli et al., 2014). Furthermore, rodent SSC possesses this activity to regenerate spermatogenesis irrespective of age of the donor (Shinohara et al., 2001, Ryu et al., 2003). A similar result was achieved with SSC from non-human primate that was cryopreserved (Hermann et al., 2012).

These results are encouraging for potential similar results in humans, but no human data are yet available.

### *B.11.6 health outcomes for male patients*

The collection of testicular tissue includes a small operation which potentially may impose surgical complications such as bleeding and infections that potentially could negatively affect the remaining testis. However, from studies in adult men and in boys undergoing unilateral testicular biopsy this does not seem to be a major problem with no long-term effects on the endocrine profile that resemble the one of the normal population (Ginsberg et al., 2010; Wyns et al., 2010; review: Picton et al., 2014).

Potential negative effects on the future fertility of boys who have had a testicular biopsy taken in childhood have not yet been clarified and require long-term follow-up studies. However, the limited amount of tissue taken and the low frequency regarding post-operative complications indicate that excision of testicular tissue in itself should not cause a significant impairment of fertility (review: Picton et al., 2014).

## **B.12 Detail the obstetric outcomes (risks and/or benefits to health) where applicable, including (but not limited to):**

### *B.12.1 observed damage to the oocyte or embryo*

The question of whether there should be any trans-generation effects on oocytes and embryos in girls born of mothers who became pregnant as a result of transplantation of frozen/thawed ovarian tissue is currently unknown. Around half of all the 35 reported children born from this procedure are girls (Macklon et al., 2014; Donnez et al., 2014). However, the oldest girl is just reaching her teenage years and the final evidence will need to await that these girls reach adulthood and start to have their own children. However, there is no reported information that suggests this should be an issue.

### *B.12.2 neonatal/infant complications*

From a recent account of babies born following transplantation of frozen/thawed ovarian tissue the total number was 35 children, who have all been reported healthy and in good conditions (Macklon et al., 2014; Donnez et al., 2014). The average weight and length of gestation are within normal limits (Macklon et al., 2014). There have been no other reported complications of neonatal nature or to the infants.

### *B.12.3 chromosomal abnormality*

There have been no specific studies looking into the chromosomal constitution of infants born from having frozen/thawed ovarian tissue transplanted and for instance infants born in Denmark have received no special attention in this regard. Further it is noticeable that there has been no report on the contrary, which is likely to suggest that this is not an issue different from the rest of the population.

### *B.12.4 congenital malformations (i.e. birth defects)*

There has been no specific report addressing this question, but as for B.12.3 there has been no report on the contrary, which is likely to suggest that this is not an issue different from the rest of the population.

#### *B.12.5 child development (physical, psychomotor and cognitive)*

Less than half a dozen children are above the age of six years and it is too early to make firm conclusions on this question. As for the Danish children there have been no adverse observations of children with regard to their physical, psychomotor and cognitive abilities that could relate to the fact that they result from grafted frozen/thawed ovarian tissue.

#### *B.12.6 psychological outcomes for child and family*

Again this is probably too early to draw any conclusions. The Danish family where the mother have delivered three children after having had Ewing sarcoma and tissue frozen and grafted have on several occasion been in television programs in Denmark and internationally, where she and her husband indeed have expressed that they are very happy about the procedure for them and their family.

#### *B.12.7 epigenetic disorders (i.e. imprinting)*

No information of this is available at the moment. To my knowledge there have been no studies on the epigenetic profiles of these children.

#### *B.12.8 maternal outcomes (including complications)*

This group of women does not run a higher risk of getting pregnant as compared to normal women. However, pregnancies should in a number of cases be categorised as high-risk obstetrical pregnancies. For instance women who during the course of treatment for their cancer disease have experienced irradiation towards their pelvis may have a uterus which is less capable of carrying a pregnancy to term. In Denmark it has recently been reported that a woman had an abortion in gestational week 19 probably due to the fact that she had received irradiation to the pelvis (Klüver Jensen et al., 2014). Other examples may include women who previously had a cervical cancer in which treatment did not include removal of the uterus.

### **B.13 Indicate if the use of cryopreserved ovarian and testicular tissue introduces any medicines to be used in a new way (if it involves a new medicine it will have to go to the Health Research Council of New Zealand's Standing Committee on Therapeutic Trials (SCOTT)). If yes, please address the following:**

#### *B.13.1 toxicity and B.13.2 interactions*

Currently ovarian tissue is transplanted without using any medicines and left to recover function spontaneously (testicular tissue has not yet been transplanted). Subsequently, the patient may need to undergo controlled ovarian stimulation as in conventional IVF treatment in order to conceive, but this does not introduce medical preparations, which is not routinely used for this purpose by other patients.

However, it can be expected that new drugs used to cause activation of resting follicles into the growing phase will be introduced in the foreseeable future. At this point in time a Japanese group headed by Professor Nao Suzuki have successfully undertaken a series of experiments in which the resting follicles are activated in the laboratory before being grafted back to the patient (Kawamura et al., 2013). The first baby following this in vitro

activation (IVA) procedure has been born. This procedure involves treatment with the substance bpV (hopic), a PTEN enzyme inhibitor, and with a substance 740YP, a PI3K stimulator, which collectively causes resting follicles to enter into the growing phase and will subsequently increase the fertility potential of the individual but also a faster burn-out of ovarian activity. The use of these and other drugs with a similar effect are likely to require approval by the Health Research Council of NZ's standing committee on Therapeutic trials.

*Testicular tissue:* One strategy for utilising stored testicular tissue involves propagation of SSC present in the tissue in vitro with a subsequent grafting of the stem cells to the patient (Valli et al., 2014). This approach may allow the patient to re-establish spermatogenesis on his own and regain fertility. However, all the in vitro procedures including media and exposure to growth factors etc. will need to undergo thorough testing for toxicity and potential effect on the progeny. Another alternative for the use of SSC is to in vitro perform spermatogenesis and use the haploid germ cells for procreation. This has now been successfully applied to mice (Sato et al., 2011). If this becomes the method of choice a thorough evaluation of the safety of using in vitro produced germ cells for procreation in humans is required.

*B.13.3 long term effects of medications (where possible, include comment on the status of the medications in New Zealand).*

Even if all the testing mentioned in B13.1 and B13.2 doesn't show any immediate adverse effects long term follow up studies will be needed in which the children born as a result of such new procedures should be followed for a number of years. However, this will be an international endeavour as it is for children conceived following conventional IVF and ICSI treatment and since NZ haven't started these treatments right now (December 2014) it will probably not impinge on NZ.

#### **B.14 Indicate if other treatment (e.g. for cancer) might be delayed as a result of using cryopreserved ovarian and testicular tissue.**

Cryopreservation of both ovarian and testicular tissue may be performed on a very short notice. Clinical practice in Denmark often involves that cryopreservation is performed on one or a few days' notice and is certainly one of the advances of this method for fertility preservation as compared to freezing of mature oocytes or embryos that will require a period of at least two weeks. So cancer treatment may potentially be postponed a few days which, however, most clinicians find acceptable. So this so short delay is not considered to worsen prognosis from the cancer treatment.

In breast cancer patients' treatment is usually not as urgent as with other cancers and a period allowing controlled ovarian stimulation and oocyte retrieval to be performed may be an option.

Grafting is only considered when the patient is well and has been disease free for often at least one or two years. As a consequence, there have been no reports that indicate that other treatment should be delayed in connection with using cryopreserved gonadal tissue.

**B.15 Indicate whether any associated risks may occur as part of the surgical intervention carried out in the use of these procedures.**

A surgical procedure always carries a risk, albeit low, of unwanted side effects. In the worst case scenario it may be fatal. However, there is no published literature to suggest any associated risks as a consequence of the surgical intervention carried out with either excising tissue or grafting frozen/thawed tissue. In Denmark it is now common practise that patients return home the same day as either the excision of the tissue is performed or that the grafting procedure has taken place.

However, on a few occasions patients have been considered too sick to undergo an operation for harvest of ovarian tissue. These patients did either not receive the procedure or it was possible to do it on a later date when they had improved to better condition, perhaps after a few rounds of chemotherapy, that potentially reduced the pool of viable follicles.

In connection with harvest of testicular tissue there is no valid information available.

**B.16 Indicate the potential age range for these treatments.**

*Ovarian tissue:* Currently the applied upper age range for offering ovarian tissue cryopreservation is normally 35 years of age (Rosendahl et al., 2011; Wallace et al., 2014) or mid-thirties depending on the biological age of the woman. If she on ultrasound shows more small antral follicles than her chronological age predicts she may be offered the cryopreservation (Rosendahl et al., 2011). This upper age limit is arbitrary chosen and reflects that the number of resting follicles at this age is likely to reach a critical low number after grafting. This reflects the fact that only a fraction of the follicles will actually regain function upon grafting, and therefore the pool of follicles that will regain activity will only provide the woman with a limited chance for conception. However, one Danish patient had tissue excised at the age 38 and when this tissue was subsequently grafted she actually became clinically pregnant but had an early abortion. In one centre in Lille, France the upper age limit is 38 years and for women aged 39–40 it is considered (Decanter C., personal communication).

Further, if IVA as described in section B13.1 and B13.2 will be clinically accepted, this procedure is likely to advance the upper age limit, since it will be possible to recruit follicles prior to grafting, which is likely to result in a burst of developing follicles, which potentially could increase fertility shortly after being grafted.

The lower age limit is basically the new born baby, where the ovaries do contain the most follicles. In Denmark the youngest patient was 7 months old and more than 25 patients less than 5 years of age have had ovarian tissue cryopreserved (Rosendahl et al., 2011).

*Testicular tissue:* The upper age range is normal puberty after which the boy is normally able to produce a semen sample, which will exclude excision of testicular tissue. If the boy is in the early years of puberty electro-stimulation may be attempted in order to retrieve mature sperm cells. The lower age limit is as for ovarian tissue the new born baby. A special category of patients are those with cryptorchidism (testis have not descended into the scrotum but remain in the abdomen shortly after birth) who from a clinically judgement may

be considered at risk of becoming permanent infertile. Often the testes are operated into the scrotum early in life and in connection with the operation a testicular biopsy is taken anyway and part of it may be taken for cryopreservation (Kvist et al., 2005).

**B.17 Indicate if the use of cryopreserved ovarian and testicular tissue can increase the risk of other disease (e.g. cancer).**

There are currently no reports to suggest that transplanted gonadal tissue as such should increase the risk of other diseases. Obviously the great concern is whether the original disease that the patient suffered from will be harboured in the frozen tissue and cause relapse upon grafting, but this issue has been described above.

In the worst case scenario the implanted tissue itself could potentially cause a malignancy as for instance ovarian cancer. However, information from Denmark demonstrates that now fifteen women have had tissue transplanted for more than five years there are currently no signs of any relapse due to the ovarian tissue in any of these women (Rosendahl et al., 2008; Schmidt et al., 2011).

Further potential malignant cell transformation was also not reported in a series of transplantations from Brussels and Valencia including 60 patients (Donnez et al., 2013). Although the precise time period of implantation was not provided in this study the first transplantation was performed in year 2003 and several of the cases have had tissue transplanted for more than five years (Dolman et al., 2013).

Collectively, there is presently no information to suggest that the freezing procedure in itself should impose any change in the tissue that potentially could lead to unwanted side-effects upon grafting.

**C. Information from animal studies (review if applicable)**

**C.18 Indicate if the use of cryopreserved ovarian and testicular tissue has been investigated in animals. If so, please specify what species and address clauses 20-22 for each species.**

Suzuki and co-workers reported on vitrification of ovarian tissue from the non-human primate cynomolgus monkeys (Suzuki et al., 2012). In six of seven animals hormone cycles were restored in a mean of 126 days following transplantation. Oocyte retrieval from two monkeys after transplantation of the ovarian cortex yielded a total of nine oocytes of which six fertilized after ICSI, but ETs did not lead to any pregnancies (Suzuki et al., 2012).

A study from China focussed on the effect of the size of the baboon ovarian tissue for successful vitrification (Lu et al., 2014). They found that smaller pieces of tissue around 1.0–1.5 mm in diameter did significantly better than pieces with a larger diameter (i.e. 2.0 mm) based on various parameters including morphologically normal primordial follicles and the follicles expressing BAX protein after vitrification–warming, but also oestradiol levels in the culture supernatants were measured.

### *Testicular:*

Sato and co-workers studied production of functional sperm in cultured neonatal mouse testes (Sato et al., 2011). They showed that neonatal mouse testes which contain only gonocytes or primitive spermatogonia as germ cells can produce spermatids and sperm in vitro with serum-free culture media. Spermatogenesis was maintained over 2 months in tissue fragments positioned at the gas–liquid interphase. From a total of 23 and 35 oocytes for sperm injection, respectively, seven and five live offspring were delivered. This small scale study reviled similar efficacy to that of in vivo-generated sperm cells. Further the reproductive capacity was examined by brother–sister mating, demonstrating that all four males and eight females were fertile. The authors concluded that the obtained spermatids and sperm resulted in healthy and reproductively competent offspring through ICSI. In addition, neonatal testis tissues were cryopreserved and, after thawing, showed complete spermatogenesis in vitro. Further, this method could with additional refinements serve as a platform for future clinical application as well as mechanistic understanding of spermatogenesis.

Collectively, if results from this mouse study can be replicated in man, it may constitute a viable approach to clinical utility of frozen testicular samples.

### **C.19 Specify the number of animals studied where cryopreserved ovarian and testicular tissue has been investigated for reproduction.**

This section mainly focuses on studies involving tissue from non-human primates. In connection with human applications non-human primates generates the most valid information, whereas many rodents and other test animals provide data that may be difficult to translate into use in a clinical setting.

A study from Belgium studied vitrification of ovarian biopsies from five adult baboons with subsequent grafting for five months (Amorin et al., 2013). Following grafting follicular development took place and corpora lutea were observed. However, the authors conclude that long term studies are required to validate its use in connection with humans (Amorin et al., 2013).

A study from Germany compared vitrification with slow-freezing of human ovarian tissue from 15 patients (Isachenko et al., 2009). The study used relatively small pieces of ovarian cortex for and evaluated the viability of the tissue with various different end-points and concluded that For cryopreservation of human ovarian tissue, conventional freezing is more promising than vitrification, because of higher developmental potential (Isachenko et al., 2009).

Collectively, these studies indicate that human ovarian tissue also may be cryostored after vitrification with surviving follicles. However, a comparison to the most widely used method of slow-freezing (which is the method almost all children conceived from this procedure has used) is difficult because no quantitative measures exist for evaluation of the number of viable follicles present the tissue following thawing.

## **C.20 Outline the efficacy of using cryopreserved ovarian and testicular tissue for:**

### **Ovarian tissue:** *C.20.1 fertilization rates and C.20.3 embryo development rates*

Obviously this information is only available for those patients who undergo IVF/ICSI procedures. Now it turns out that many women conceive spontaneously without the use of IVF/ICSI after being grafted with tissue.

However, for those who do undergo ART, there are now several reports that demonstrate difficulties in recruiting more than just a few follicles in patients transplanted with frozen/thawed ovarian tissue. Furthermore, the developmental potential of retrieved oocytes is low and the overall rate of embryo formation is low. In the largest reported series from Denmark a total of 56 treatment cycles resulted in the development of 76 pre-ovulatory follicles that yielded 49 oocytes that subsequently resulted in 18 fertilised oocytes of which 16 were transplanted (Schmidt et al., 2010). In Brussels they have reported that 21 IVF attempts in four women resulted in a total of 16 oocytes of which 5 subsequently developed into embryos (Dolmans et al., 2009). In Spain one patient who subsequently became pregnant underwent four stimulation cycles in which a total of 18 oocytes were collected and vitrified prior to being used (Sanchez-Serrano et al., 2009). Collectively, these data suggest that IVF in patients with transplanted cryopreserved ovarian tissue result in a low number of retrieved oocytes with subsequent low pregnancy rates (Schmidt et al., 2010).

### *C.20.2 survival rate of the oocytes and sperm following re-implantation of ovarian and testicular tissue*

It is not yet possible to detect the number of follicles present in ovarian cortex in a non-destructive manner. Further the density of follicles in ovarian cortex is extremely variable with differences of more than three orders of magnitude in between pieces prepared for transplantation (Schmidt et al., 2003). Therefore it is not possible to determine the number of transplanted follicles and it is not possible to determine the number of follicles that survive the first days in which the tissue is without proper blood supply and subsequent becomes available to the woman.

Collectively, there is no solid information of the survival rate of follicles following transplantation in humans.

### *C.20.4 pregnancy rates (please compare to use of fresh mature gametes) and C.20.5 live birth rates (please compare to use of fresh mature gametes)*

In contrast to IVF/ICSI treatments where a positive pregnancy test with certainty is determined two weeks following retrieval of oocytes (and a live birth nine months later) the issues around ovarian tissue is different. Whereas patients receiving IVF/ICSI treatment have had a problem in the past (often for years) and approach a physician to solve their problem, patients receiving ovarian tissue are facing the future and these two patient categories turn out to be completely different. The life situation of patients transplanted with ovarian tissue may change; it is not uncommon that cancer survivors have a divorce. The following information illustrate the difficulty in making a direct comparison between pregnancy rates of ART techniques and by grafting ovarian tissue.



1. In Denmark a relatively large fraction of the women are just young women who want to avoid menopause and don't yet have a pregnancy wish. In Denmark we have actually witnessed one legal abortion after woman had frozen/thawed ovarian tissue transplanted. She was in the middle of breaking up with her boyfriend and didn't want to give birth to his child (Greve et al., 2010).
2. Some of the women having tissue grafted may not be completely devoid of endogenous ovarian activity, but they are perhaps having irregular menstrual cycles (and a low ovarian reserve) and experience difficulty in conceiving. They request augmentation of their pool of follicles by grafting frozen/thawed tissue, which obviously makes it difficult to determine whether the subsequent pregnancy derives from the endogenous stores of follicles or from the transplanted tissue.
3. In patients who suffered from a cervical cancer their uterus may have been removed with the consequence that they cannot carry a pregnancy themselves. If they have tissue grafted a surrogacy mother is needed to carry the pregnancy once mature oocytes have been derived from the transplanted tissue. This obviously puts further constraints on how to calculate the success rate of having tissue transplanted. In Denmark, one woman in this situation has had eight embryos created outside Denmark for transplantation to a surrogate mother (which is illegal in Denmark) (Strauss et al., 2014). However, she did not get pregnant.
4. One Danish woman is married to a husband who has a cultural background where men by definition cannot be infertile. He has hardly any sperm cells in his ejaculate and requires ICSI to fertilise an oocyte. However, the couple maintain that they want to conceive without assistance, which obviously will be difficult and adds to the complexity of calculating a valid estimate of pregnancy chances with this procedure.
5. In addition, pregnancies often are a result of natural conception without employing ART procedures and therefore make a direct comparison to IVF procedures difficult.
6. Furthermore pregnancy rates may constantly change as long as the tissue remains active and a rolling and increasing pregnancy rate is observed.
7. The woman normally will achieve menstrual cycles four to five months after grafting the tissue and should only count in the statistics after the tissue has become active.

The above illustrate that it is difficult to determine the nominator and the denominator going into the equation of calculating the pregnancy rate. Basically it does not make sense to make direct comparison of pregnancy chances between ART procedures and by replacing ovarian tissue.

The below table shows the current results of the 43 women in Denmark who have had frozen/thawed ovarian tissue grafted (Klüver-Jensen et al., 2015 (in preparation)). This result is likely to change in the future when more women from this group becomes pregnant or experience an exhausted ovarian function.

	No.	No.	%
<b>All women</b>			
No. pos. hCH per woman transplanted	22	43	51
No. clinical pregnancies per woman transplanted**	16	43	37
No children born per woman transplanted	8 (4)*	43	19 (28)*
<b>Women who wanted to become pregnant***</b>			
No. pos. hCH per woman transplanted	22	30	73
No. clinical pregnancies per woman transplanted**	16	30	53
No children born per woman transplanted	8 (4)*	30	27 (40)*
<b>Calculated per women</b>			
No. women (pos. hCG) per total who wanted to conceive	16	30	53
No. women (clinical preg.) per total who wanted to conceive**	12	30	40
No women with children per total who wanted to conceive	5 (8)*	30	20 (40)*

\* Currently three women are pregnant with four children.

\*\* The group of clinical pregnancies include two women who had a legal abortion and one woman who had rupture of the membranes in week 19 and lost the baby.

\*\*\* Women not included in this group are: women who only wanted to alleviate menopausal symptoms, women who suffered from cervical cancer, women who divorced their partner, women who had tissue transplanted less than six months ago.

### C.20.6 diagnostic accuracy of the procedure

Currently our information of the diagnostic accuracy of the procedure is low or basically absent. We cannot predict who will conceive and who will not. Age at cryopreservation is a likely to be a critical parameter that may predict the chances of conceiving. The amount of tissue transplanted will reflect the pool of follicles available for supporting follicular development and is also likely to reflect the chance of conception (Klüver-Jensen et al., 2015 (in preparation)).

Our current knowledge of using testicular tissue for restoring fertility is too limited to answer any of the above questions.

## C.21 Detail any risks to health of using cryopreserved ovarian and testicular tissue, including (but not limited to):

### C.21.1 any potential side effects

Any operations involving complete anaesthesia always include a risk. The operations needed in order remove ovarian tissue and to replace tissue is, however, considered low-risk operations and no major complications from the operations have been reported. Women will usually not stay overnight at the hospital for neither excision of tissue nor for transplantation. A similar consideration may be applied for men having testicular tissue excised and grafted.

Provided that tissue samples – either ovarian or testicular tissue – is maintained in liquid nitrogen and do not experience suboptimal handling during the storage period there is no information to suggest that there is any measurable deterioration (Picton et al., 2014). For instance, children have been born from semen stored for over 28 years (Feldschuh et al., 2005) and in Denmark we have transplanted ovarian tissue 10 years after cryopreservation with subsequent function.

#### *C.21.2 health outcomes for both female and male subjects*

Apart from having a relapse as a result of having malignant cells grafted potential negative health outcome could include the development of malignancies in the grafted tissue itself, for instance development of ovarian cancer. Although long term follow up studies will be required and is still missing, current results do not suggest any increased risk of other malignancies. On the contrary it may be argued that women who would otherwise be menopausal for a considerably younger age than the normal age of menopause may benefit from having menstrual cycles as a result of the implanted ovarian tissue and may potentially delay onset of osteoporosis and other menopausal related symptoms.

#### *C.21.3 ongoing development of offspring born as a result of the procedure*

There is currently no information to suggest that these children should be any different from children conceived the normal way. However, the number of offspring is limited and further research is required to provide answer to this question.

### **C.22 Detail the obstetric outcomes (risks and/or benefits to health), including (but not limited to):**

#### *C.22.1 neonatal/infant complications and C.22.2 chromosomal abnormality and C.22.3 congenital malformations (i.e. birth defects)*

These questions are obviously highly interesting; however, the main conclusion is that there are no solid data available. However, a recent study evaluated 26 children born from having frozen/thawed ovarian tissue transplanted and the gestational age was on average 38, 5 weeks for singletons and 35 weeks for two sets of twins. The birth weight was on average 3.172 grams for singletons and 2516 grams for the two sets of twins (Macklon et al., 2014). These figures do not differ from normal pregnancies.

At this point in time, the grafted women probably represent a population of women who are at a higher risk of experiencing a pregnancy with obstetrical problems, for instance they may have had irradiation towards the pelvis. No information have been published to suggest that chromosomal malformations should be prevalent in these children nor have there been published information that suggests that these children should be any different from children conceived “naturally”.

#### *C.22.4 offspring development (physical, psychomotor and cognitive)*

There is no information available on offspring development.

#### *C.22.5 epigenetic disorders (i.e. imprinting)*

There is no information available on the epigenetic profile of offspring.

## **D. Feasibility of undertaking these procedures in New Zealand**

### **D.23 Comment on the feasibility of these procedures being undertaken in New Zealand i.e. the requirement for any specific clinical capability and other associated medical processes.**

There is no reason why these procedures could not be implemented in NZ. The equipment necessary for performing the procedure will already be available and is low-cost anyway. However, it is suggested that a transport system for tissue prior to cryopreservation as developed in Denmark (Schmidt et al., and now implemented in a number of European countries should also be the model of choice in NZ. Here one laboratory carries out the actual cryopreservation procedure for the whole country. The infrastructure in NZ will allow the tissue be transported from anywhere in the country to one of the major cities within a few hours. This will allow one centre to specialise in these methods, to get the necessary expertise with a sufficient number of patients, be able to implement quality control systems instead of many centres doing a few cases and nobody obtaining good experience and expertise.

The annual activity in Denmark is around 70 cases of ovarian tissue with a population of around five million inhabitants and it is estimated that a similar number of cases will be relevant in NZ. In Denmark and other places there are on a regular basis hands-on courses where participants become educated in performing these procedures. Actually, already one person from NZ has attended one such course in Denmark.

### **D.24 Outline any procedural risks identified in countries where these procedures have being approved and undertaken.**

With human involvement there is always a risk of procedural mistakes. To illustrate potential risks one incidence will highlight this issue. For transportation of excised tissue crushed ice with a temperature of around zero degrees was by mistake replaced with dry ice, which has a temperature of  $-80$  degrees. Upon arrival the tissue was deep frozen and none of the follicles survived. Therefore the whole chain of people involved needs a thorough training and education. It is important to realise that fertility preservation is a multi-disciplinary effort with a number of expertise's involved and a good outcome is dependent on everyone doing the right and optimal procedure.

There have been no reports on procedural risks up until now. However, even considering the ultimate mistake, replacing tissue to a woman that belongs to someone else, is likely to have a limited effect. The tissue will be considered as an organ by the host and if tissue at random is grafted from one person to the other the likelihood of it being accepted and not rejected by the immune system is very small.

## **E General**

### **E.25 Specify and detail any additional information related to the risks or benefits to health of using cryopreserved ovarian and testicular tissue, not canvassed in the above clauses, that should be considered when making an assessment of the risks and benefits to health of the use of re-implanted ovarian tissue (or**

**oocytes matured from cryopreserved ovarian tissue) and testicular tissue that have previously undergone cryopreservation.**

There are an estimated 200 women who have had frozen/thawed ovarian tissue grafted and the number of women who experienced a relapse is reassuringly low and the relapse is furthermore most likely not related to the ovarian tissue in these cases. However, potential relapses are properly under-reported and more long term studies are required to substantiate the low rate of relapse.

The psychological impact of having gonadal tissue frozen is important for some women facing an often harsh gonadotoxic treatment. The fact that somebody has considered their fertility later on when they have withstood a demanding treatment is reassuring. Although a French study has addressed these issues more information on this subject will be of interest (Trèves et al., 2011; 2014).

A recent study showed that more than 80 percent of women who have had tissue cryopreserved for five years wanted to keep their tissue stored for potential use in the future (Macklon et al., 2014). Most of the remaining patients had already completed their family and wanted to dispose their tissue.

As described above, the fact that the ovarian tissue in addition to providing fertility also has the capacity to produce female sex steroids (i.e. oestradiol and progesterone) is likely to raise a number of other issues related to risks and benefits to health in the coming years. This is likely to include a discussion of prevention of osteoporosis and cardiovascular diseases on one hand and the discussion of potentially increasing the risk of other cancer like for instance breast cancer.

The main indication for cryopreserving ovarian and testicular tissue is to restore fertility subsequently. Therefore the cost of whole chain of events needs to be considered when implementing this procedure. The cost related to grafting tissue needs to be considered prior to excising tissue. There is for instance no point in excising ovarian tissue from a young woman or a young girl for fertility purposes if she in adult life cannot carry a pregnancy. She may have uterus removed in case of a cervical cancer or she may need so much irradiation that the uterus is unlikely to be able to carry a pregnancy. There may be other obstetrical or other considerations that may advise against the woman becoming pregnant, when she has overcome the cancer treatment. Obviously, the above discussion of the use of ovarian tissue for steroid production may impact on these considerations.

Further, if the young woman is unlikely to afford the cost of having tissue transplanted and if she will have no means of paying for potential ART procedures, it may not be worthwhile to excise the tissue in the first place. At least information on the conditions and cost for the subsequent use of the tissue should be made absolutely clear before embarking on the procedure on the first hand.

In many countries there are running costs for maintaining the tissue cryostored – and if the tissue is taken from a young girl it may take many years before she will return to have it transplanted and is the patient capable of sustaining this long term cost needs to be considered.

## **E.26 Outline any known long-term follow-up studies presented to date and any planned for the future**

In Denmark a follow up study on the fertility of women who have had one ovary excised has recently been published (Schmidt et al., 2013). The study included women above the age of 18 years and had removal of tissue performed more than 2 years ago. The participation rate was 78 % leaving a total of 143 participating women. The mean follow-up time was 58 months. Eighty percent confirmed they wanted to use the tissue if necessary. Of women who did not become sterile from the cancer treatment 57 women had attempted to become pregnant and 41 (72%) succeeded, while 84 did not yet have pregnancy wish (23 % were still on medication or advised against getting pregnant).

As mentioned above the Danish cohort of women grafted is currently being prepared for publication and will probably appear in the literature in year 2015. Similar publications from other groups are also now in preparation.

There are no long term studies published on women having ovarian tissue transplanted. These women are followed continuously and long-term follow-up studies are being performed, but information is just not very solid at this point in time.

## **E.27 Comment on the quality of the published research**

The quality of the published studies is generally good and state-of-the-art techniques and approaches have been used. However, fertility preservation is a totally new field and the current technology of assessing for instance safety is inadequate and does not provide solid answers. For cancers the minimal infectious number of cells is unknown, in some cancers a few cells are probably enough to cause relapse, whereas in others substantially higher number of cells is required. Basically follow-up studies on transplantation to women who previously suffered from a cancer will provide more solid answers to safety issues.

Almost any step in these procedures has been developed based on empirical approaches and will be subjected to improvements in the coming years especially the transplantation methods. Many of the published studies on women having tissue grafted are observational studies, because it is not ethical acceptable to do randomised controlled studies, in which the control arm will receive a sham operation and have no tissue transplanted.

The field is characterised by a few large centres which drive development and where the main progress derives from. However, many centres want to get on the bandwagon and the number of published reviews almost exceeds the number of original studies. However it does reflect a huge worldwide interest and increase in activity of this field.

## **E.28 List references to all published and peer reviewed research used in the report**

NB: web-based references should only be used when absolutely necessary and should indicate the date of access. All references should conform to the Vancouver system i.e. authors, year of publication, full title, Journal title in full, volume and inclusive pages. References 'in print' or 'epub' should provide the journal information and the doi.

## References

1. Abir R, Feinmesser M, Yaniv I et al.: Occasional involvement of the ovary in Ewing sarcoma. *Hum. Reprod.* 2010;25:1708-1712.
2. Abir R, Aviram A, Feinmesser M, Stein J, Yaniv I, Parnes D, Ben-Haroush A, Meiorow D, Rabizadeh E, Fisch B. *Reprod Biomed Online.* 2014;28:255-60.
3. Amorim CA, Jacobs S, Devireddy RV, Van Langendonck A, Vanacker J, Jaeger J, Luyckx V, Donnez J, Dolmans MM. Successful vitrification and autografting of baboon (*Papio anubis*) ovarian tissue. *Hum Reprod.* 2013;28:2146-56.
4. Amorim CA, Curaba M, Van Langendonck A, Dolmans MM, Donnez J. Vitrification as an alternative means of cryopreserving ovarian tissue. *Reprod Biomed Online.* 2011;23:160-86.
5. Amorim CA, Dolmans MM, David A, Jaeger J, Vanacker J, Camboni A, Donnez J, Van Langendonck A. Vitrification and xenografting of human ovarian tissue. *Fertil Steril.* 2012;98:1291-8.e1-2.
6. Andersen CY, Silber SJ, Berghold SH, Jorgensen JS, Ernst E. Long-term duration of function of ovarian tissue transplants: case reports. *Reprod Biomed Online.* 2012;25:128-32.
7. Andersen CY, Kristensen SG, Greve T, Schmidt KT. Cryopreservation of ovarian tissue for fertility preservation in young female oncological patients. *Future Oncol.* 2012;8:595-608.
8. Bastings L, Beerendonk CC, Westphal JR, Massuger LF, Kaal SE, van Leeuwen FE, Braat DD, Peek R. Autotransplantation of cryopreserved ovarian tissue in cancer survivors and the risk of reintroducing malignancy: a systematic review. *Hum Reprod Update.* 2013a;19:483-506.
9. Bastings L, Beerendonk CCM, Westphal JR, Braat DDM, Peek R. Cryopreservation and Autotransplantation of Ovarian Tissue in Cancer Patients: Is It Safe? *J Adolesc Young Adult Oncol* 2013b;2:31-34.
10. Bergh J, Wiklund T, Erikstein B, Lidbrink E, Lindman H, Malmström P, Kellokumpu-Lehtinen P, Bengtsson NO, Söderlund G, Anker G, Wist E, Ottosson S, Salminen E, Ljungman P, Holte H, Nilsson J, Blomqvist C, Wilking N. Tailored fluorouracil, epirubicin, and cyclophosphamide compared with marrow-supported high-dose chemotherapy as adjuvant treatment for high-risk breast cancer: a randomised trial. Scandinavian Breast Group 9401 study. *Lancet.* 2000;356:1384-91.
11. Bjelland EK, Wilkosz P, Tanbo TG, Eskild A. Is unilateral oophorectomy associated with age at menopause? A population study (the HUNT2 Survey). *Hum Reprod.* 2014;29:835-41.
12. Bittinger SE, Nazaretian SP, Gook DA, Parmar C, Harrup RA, Stern CJ. Detection of Hodgkin lymphoma within ovarian tissue. *Fertil Steril.* 2011;95:803.
13. Borgstrom B, Hreinsson J, Rasmussen C, Sheikhi M, Fried G, Keros V, Fridstrom M, Hovatta O. Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab* 2009;94:74–80.
14. Brook PF, Radford JA, Shalet SM, Joyce AD, Gosden RG. Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation. *Fertil Steril* 2001;75:269–74.
15. Campbell BK, Hernandez-Medrano J, Onions V, Pincott-Allen C, Aljaser F, Fisher J, McNeilly AS, Webb R, Picton HM. Restoration of ovarian function and natural fertility following the

- cryopreservation and autotransplantation of whole adult sheep ovaries. *Hum Reprod.* 2014;29:1749-63.
16. Directive 2011/24/EU of the European Parliament and of the Council of 9 March 2011 on the application of patients' rights in cross-border healthcare (OJ No. L88, 4.4. 2011, p.45).
  17. Dolmans M-M, Donnez J, Camboni A, Demylle D, Amorim C, Van Langendonck A, et al. IVF outcome in patients with orthotopically transplanted ovarian tissue. *Hum Reprod* 2009;24:2778–87.
  18. Dolmans MM, Marinescu C, Saussoy P, Van Langendonck A, Amorim C, Donnez J. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. *Blood.* 2010;116:2908-14.
  19. Donnez J, Martinez-Madrid B, Jadoul P, Van Langendonck A, Demylle D, Dolmans MM. Ovarian tissue cryopreservation and transplantation: a review. *Human Reprod Update.* 2006;12:519 – 35.
  20. Donnez, J., Dolmans, M. M., Demylle, D., Jadoul, P., Pirard, C., Squifflet, J., Martinez-Madrid, B. and Van Langendonck, A. (2006) Restoration of ovarian function after orthotopic (intraovarian and periovarian) transplantation of cryopreserved ovarian tissue in a woman treated by bone marrow transplantation for sickle cell anaemia: case report. *Hum Rep, 21*, 183-188.
  21. Donnez J, Jadoul P, Pirard C, Hutchings G, Demylle D, Squifflet J, Smitz J, Dolmans MM. Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. *Fertil Steril.* 2012;98:720-5.
  22. Donnez J, Silber S, Andersen CY, Demeestere I, Piver P, Meirow D, Pellicer A, Dolmans MM. Children born after autotransplantation of cryopreserved ovarian tissue. a review of 13 live births. *Ann Med.* 2011;43:437-50.
  23. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Serrano MS, Schmidt KT, et al. Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. *Fertil Steril.* 2013;99:1503-13.
  24. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Ernst E, Macklon KT, Yding Andersen C. Pregnancies and live births after ovarian tissue transplantation: the true picture. *Lancet* (in press).
  25. Ernst E, Kjærsgaard M, Birkebæk NH, Clausen N, Yding Andersen C. Stimulation of puberty in a girl with chemo – and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. *Eur. J. Cancer,* 2013;49: 911-914.
  26. Ernst EH, Vrou Offersen B, Yding Andersen C, Ernst E. Legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue due to cancer recurrence. *J Assist Reprod Genet* 2013;30:975–978.
  27. Ernst EH, Offersen BV, Andersen CY, Ernst E. Legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue due to cancer recurrence. *J Assist Reprod Genet.* 2013;30:975-8.
  28. Feldschuh J, Brassel J, Durso N, Levine A. Successful sperm storage for 28 years. *Fertil Steril* 2005 84:1017.
  29. Fertility preservation and reproduction in cancer patients. *Fertil Steril* 2005; 83:1622–28.



30. Ginsberg JP, Carlson CA, Lin K, Hobbie WL, Wigo E, Wu X, Brinster RL, Kolon TF. An experimental protocol for fertility preservation in prepubertal boys recently diagnosed with cancer: a report of acceptability and safety. *Hum Reprod* 2010; 25: 37-41.
31. Ginsberg JP. New advances in fertility preservation for pediatric cancer patients. *Curr Opin Pediatr* 2011;23:9–13.
32. Greve T, Ernst E, Markholt S, Schmidt KT, Andersen CY. Legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue. *Acta Obstet Gynecol Scand.* 2010; 89:1589-91.
33. Greve T, Schmidt KT, Kristensen SG, Ernst E, Andersen CY. Evaluation of the ovarian reserve in women transplanted with frozen and thawed ovarian cortical tissue. *Fertil Steril.* 2012;97:1394-98.
34. Greve T, Clasen-Linde E, Andersen MT, Andersen MK, Sørensen SD, Rosendahl M, Ralfkiaer E, Andersen CY. Cryopreserved ovarian cortex from patients with leukemia in complete remission contains no apparent viable malignant cells. *Blood.* 2012;120:4311-16.
35. Greve T, Wielenga VT, Grauslund M, Sørensen N, Christiansen DB, Rosendahl M, Yding Andersen C. Ovarian tissue cryopreserved for fertility preservation from patients with Ewing or other sarcomas appear to have no tumour cell contamination. *Eur J Cancer.* 2013;49:1932-8.
36. Goossens E, Tournaye H. Male fertility preservation, where are we in 2014? *Ann Endocrinol (Paris).* 2014;75:115-17.
37. Henes M, Henes JC, Neunhoeffer E, Von Wolff M, Schmalzing M, Kötter I, Lawrenz B. Fertility preservation methods in young women with systemic lupus erythematosus prior to cytotoxic therapy: experiences from the FertiPROTEKT network. *Lupus.* 2012;21:953-8.
38. Hermann BP, Sukhwani M, Winkler F, Pascarella JN, Peters KA, Sheng Y, et al. Spermatogonial stemcell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm. *Cell Stem Cell* 2012;11:715–26.
39. Hou M, Andersson M, Eksborg S, Soder O, Jahnukainen K, 2007: Xenotransplantation of testicular tissue into nude mice can be used for detecting leukemic cell contamination. *Hum. Reprod.* 22 1899-1906.
40. Isachenko V, Lapidus I, Isachenko E, Krivokharchenko A, Kreienberg R, Woriedh M, Bader M, Weiss JM. Human ovarian tissue vitrification versus conventional freezing: morphological, endocrinological, and molecular biological evaluation. *Reproduction.* 2009;138:319-27.
41. ISFP Practice Committee, Kim SS, Donnez J, Barri P, Pellicer A, Patrizio P, Rosenwaks Z, Nagy P, Falcone T, Andersen C, Hovatta O, Wallace H, Meirou D, Gook D, Kim SH, Tzeng CR, Suzuki S, Ishizuka B, Dolmans MM. Recommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. *J Assist Reprod Genet.* 2012;29:465-468.
42. Ito T. Stem cell maintenance and disease progression in chronic myeloid leukemia. *Int J Hematol.* 2013;98:641-47.
43. Jahnukainen K, Hou M, Petersen C, Setchell B, Soder O, 2001: Intratesticular transplantation of testicular cells from leukemic rats causes transmission of leukemia. *Cancer Res.* 61 706-710.

44. Janse F, Donnez J, Anckaert E, de Jong FH, Fauser BC, Dolmans MM. Limited value of ovarian function markers following orthotopic transplantation of ovarian tissue after gonadotoxic treatment. *J Clin Endocrinol Metab.* 2011;96:136-44.
45. Jadoul P, Kim SS. ISFP Practice Committee. Fertility considerations in young women with hematological malignancies. *J Assist Reprod Genet.* 2012;29:479–87.
46. Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci USA.* 2013;110:17474-9.
47. Kim SS, Lee WS, Chung MK, Lee HC, Lee HH, Hill D. Long-term ovarian function and fertility after heterotopic autotransplantation of cryobanked human ovarian tissue: 8-year experience in cancer patients. *Fertil Steril.* 2009;91:2349–54.
48. Klemp JR, Kim SS; ISFP Practice Committee. Fertility preservation in young women with breast cancer. *J Assist Reprod Genet.* 2012;29:469-72.
49. Kroman N, Jensen MB, Melbye M, Wohlfahrt J, Mouridsen HT. Should women be advised against pregnancy after breast-cancer treatment? *Lancet.* 1997;350:319-22.
50. Kvist K, Thorup J, Byskov AG, Høyer PE, Møllgård K, Yding Andersen C: Cryopreservation of intact testis tissue from boys with cryptorchidism. *Hum Reprod.* 2006;21:484-491.
51. Kyono K, Doshida M, Toya M, Sato Y, Akahira J, Sasano H. Potential indications for ovarian autotransplantation based on the analysis of 5,571 autopsy findings of females under the age of 40 in Japan. *Fertil. Steril.* 2010;93:2429-2430.
52. Lanman JT, Bierman HR, Byron RL Jr. Transfusion of leukemic leukocytes in man; hematologic and physiologic changes. *Blood.* 1950;5:1099-1113.
53. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J Clin Oncol* 2006;24:2917–31.
54. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K; American Society of Clinical Oncology. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol.* 2013;31:2500-10.
55. Lu XL, Yu J, Zhang G, Wei ZT, Li JT, Zhang JM. Effects of varying tissue sizes on the efficiency of baboon ovarian tissue vitrification. *Cryobiology.* 2014;69:79-83.
56. Macklon KT, Jensen AK, Loft A, Ernst E, Andersen CY Treatment history and outcome of 24 deliveries worldwide after autotransplantation of cryopreserved ovarian tissue, including two new Danish deliveries years after autotransplantation. *J Assist Reprod Genet.* 2014;31:1557-64.
57. Macklon KT, Ernst E, Andersen AN, Andersen CY. Cryobanking of human ovarian tissue: do women still want their tissue stored beyond 5 years? *Reprod Biomed Online.* 2014;29:452-56.
58. McCook A. A future, on ice. *Nat Med* 2013;19:958–61.
59. Oktay K, Oktem O. Fertility preservation medicine: A new field in the care of young cancer survivors. *Pediatr Blood Cancer* 2009;53:267–273.

60. Picton HM, Wyns C, Anderson RA, Goossens E, Jahnukainen E, Kliesch S, Mitchell RT, Pennings G, Rives N, Tournaye H, Van Pelt AHM, Eichenlaub-Ritter U, Van den Abbeel E, Schlatt S. A European Perspective On Testicular Tissue Cryopreservation For Fertility Preservation In Prepubertal And Adolescent Boys. *Human Reproduction Update* (in press).
61. Poirot C, Abirached F, Prades M, Coussieu C, Bernaudin F, Piver P. Induction of puberty by autograft of cryopreserved ovarian tissue. *Lancet*, 2012;379:588.
62. Practice Committee of American Society for Reproductive Medicine. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. *Fertil Steril* 2013; 100: 1214–23.
63. Radford J, Shalet S, Lieberman B. Fertility after treatment for cancer. Questions remain over ways of preserving ovarian and testicular tissue. *BMJ*. 1999;319:935-36.
64. Radford J. Restoration of fertility after treatment for cancer. *Horm Res* 2003;59 (Suppl 1):21–23.
65. Riggs BL, Melton LJ. The worldwide problem of osteoporosis: Insights afforded by epidemiology. *Bone* 1995;5:S505-S512.
66. Rives N, Milazzo JP, Perdrix A, Castanet M, Joly-Hélas G, Sibert L, Bironneau A, Way A, Macé B. The feasibility of fertility preservation in adolescents with Klinefelter syndrome. *Hum Reprod*. 2013;28:1468-79.
67. Rosendahl M, Yding Andersen C, Ernst E, Westergaard LG, Rasmussen PE, Loft A, Nyboe Andersen A: Ovarian function after removal of an entire ovary for cryopreservation of pieces of cortex prior to gonadotoxic treatment: a follow up study *Hum. Reprod* 2008;23:2475-83.
68. Rosendahl M, Ahlgren J, Andersen J, Bergh J, Blomquist C, Lidbrink E, Lindman H, Mouridsen H, Bjerre K, Andersson M. The risk of amenorrhoea after adjuvant chemotherapy for early stage breast cancer is related to inter-individual variations in chemotherapy-induced leukocyte nadir in young patients: data from the randomised SBG 2000-1 study. *Eur J Cancer*. 2009;45:3198-204.
69. Rosendahl M, Andersen MT, Ralfkiaer E, Kjeldsen L, Andersen MK, Andersen CY. Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. *Fertil Steril*. 2010;94:2186-90.
70. Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, Nyboe Andersen A, Yding Andersen C: Cryopreservation of ovarian tissue for a decade in Denmark – An overview of the technique. *Reprod Biomed Online* 2011;22:162-71.
71. Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: a review of the literature. *J Assist Reprod Genet*. 2013;30:11-24.
72. Roux C, Amiot C, Agnani G, Aubard Y, Rohrlich PS, Piver P. Live birth after ovarian tissue autograft in a patient with sickle cell disease treated by allogeneic bone marrow transplantation. *Fertil Steril* 2010;93:2413.e15-9.
73. Ryu BY, Orwig KE, Avarbock MR, Brinster RL. Stem cell and niche development in the postnatal rat testis. *Dev Biol* 2003;263:253–63.
74. Sadri-Ardekani H, Atala A. Testicular tissue cryopreservation and spermatogonial stem cell transplantation to restore fertility: from bench to bedside. *Stem Cell Res Ther*. 2014;5:68.

75. Sanchez-Serrano M, Crespo J, Mirabet V, Cobo AC, Escribá M-J, Simo´n C, et al. Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. *Fertil Steril* 2010;93:268.e11–3.
76. Schmidt KL, Byskov AG, Nyboe Andersen A, Müller J, Yding Andersen C. Density and distribution of primordial follicles in single pieces of cortex from 21 patients and in individual pieces of cortex from three entire human ovaries. *Hum Reprod.* 2003;18:1158-64.
77. Schmidt KL, Ernst E, Byskov AG, Nyboe Andersen A, Yding Andersen C. Survival of primordial follicles following prolonged transportation of ovarian tissue prior to cryopreservation. *Hum Reprod.* 2003;18:2654-59.
78. Schmidt KLT, Yding Andersen C, Starup J, Loft A, Byskov AG, Nyboe Andersen A. Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer – follicular growth, steroid production and oocyte retrieval. *Reprod Biomed Online.* 2004;8:448-53.
79. Schmidt KT, Rosendahl M, Ernst E, Loft A, Nyboe Andersen A, Dueholm M, Ottosen C, Yding Andersen C: Autotransplantation of cryopreserved ovarian tissue in 12 women with chemotherapy-induced premature ovarian failure: The Danish experience. *Fertil Steril* 2011;95:695-701.
80. Schmidt KTL, Larsen EC, Yding Andersen C, Nyboe Andersen A: Risk of ovarian failure and fertility preserving methods in girls and adolescents with a malignant disease. *British J Obstet. Gynaecol.* 2010;117:163-74.
81. Schmidt KT, Andersen CY. ISFP Practice Committee. Recommendations for fertility preservation in patients with lymphomas. *J Assist Reprod Genet.* 2012;29:473–77.
82. Schmidt KT, Nyboe Andersen A, Greve T, Ernst E, Loft A, Yding Andersen C. Fertility in cancer patients after cryopreservation of one ovary. *Reprod Biomed Online.* 2013;26:272-79.
83. Shapira M, Raanani H, Cohen Y, Meirou D. Fertility preservation in young females with hematological malignancies. *Acta Haematol.* 2014;132:400-13.
84. Shinohara T, Orwig KE, Avarbock MR, Brinster RL. Remodeling of the postnatal mouse testis is accompanied by dramatic changes in stem cell number and niche accessibility. *Proc Natl Acad Sci U S A* 2001;98:6186–91.
85. Silber SJ. Ovary cryopreservation and transplantation for fertility preservation. *Mol Hum Reprod* 2012; 18: 59–67.
86. Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, et al. First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. *Hum Rep* 2013;28:2996-99.
87. Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, Jobling T. Delivery of twins following heterotopic grafting of frozen-thawed ovarian tissue. *Hum Reprod.* 2014;29:1828.
88. Stoop D, Cobo A, Silber S. Fertility preservation for age-related fertility decline. *Lancet,* 2014;384:1311-19.
89. Storeng R, Abyholm T, Tanbo T. Cryopreservation of ovarian tissue . *Tidsskr Nor Laegeforen.* 2007;127:1045-48.
90. Sørensen SD, Greve T, Timmermans Wielenga V, Wallace WHB, Yding Andersen C. Safety considerations for transplanting frozen/thawed ovarian tissue to restore fertility in patients who have recovered from Ewing sarcoma. *Future Oncol.* 2014;10:277-283.

91. Tournaye H, Dohle GR, Barratt CL. Fertility preservation in men with cancer. *Lancet*, 2014;384:1295-301.
92. Trèves R, Grynberg M, Parco SI, Finet A, Poulain M, Fanchin R. Female fertility preservation in cancer patients: an instrumental tool for the envisioning a postdisease life. *Future Oncol*. 2014;10:969-74.
93. Trèves R, Grynberg M, Hesters L, Fanchin R, Frydman R. Fertility preservation in cancer women: a multidisciplinary approach]. *J Gynecol Obstet Biol Reprod (Paris)*. 2011;40:487-91.
94. UK – Department of Health: [https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/252940/Cross\\_Border\\_Healthcare\\_Information.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/252940/Cross_Border_Healthcare_Information.pdf)
95. Valli H, Phillips BT, Shetty G, Byrne JA, Clark AT, Meistrich ML, Orwig KE. Germline stem cells: toward the regeneration of spermatogenesis. *Fertil Steril*. 2014;101:3-13.
96. von Schoultz E, Johansson H, Wilking N, Rutqvist LE. Influence of prior and subsequent pregnancy on breast cancer prognosis. *J Clin Oncol*. 1995;13:430-4.
97. Wallace WH, Smith AG, Kelsey TW, Edgar AE, Anderson RA. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. *Lancet Oncol*. 2014;15:1129-36.
98. Wyns C, Curaba M, Vanabelle B, Van Langendonck A, Donnez J. Options for fertility preservation in prepubertal boys. *Hum Reprod Update* 2010;16:312-328.
99. Wyns C, Curaba M, Petit S, Vanabelle B, Laurent P, Wese JF, et al. Management of fertility preservation in prepubertal patients: 5 years' experience at the Catholic University of Louvain. *Hum Rep* 2011;26:737–47.
100. Yasui T, Hayashi K, Mizunuma H, Kubota T, Aso T, Matsumura Y, Lee JS, Suzuki S. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas*. 2012;72:249-55.
101. Yding Andersen C, Ernst E, Bærentzen S, Birkebæk NH, Clausen N. No malignancy detected in surplus ovarian tissue from a former Ewing sarcoma patient who experienced relapse four years after being grafted with frozen/thawed ovarian tissue. *J Assist Reprod Genet*. 2014;31:1567-68.
102. Yding Andersen C. Possible malignant cell contamination in transplanted ovarian tissue. International Society for Fertility Preservation, News letter, 2014;2:2. [www.isfp-fertility.org/](http://www.isfp-fertility.org/)
103. Yding Andersen C and Kristensen SG. The increased life expectancy in women is concurrent with long periods in menopause: do the ovaries themselves hold promise of tackling the sequelae of menopause? *RBMOnline* (submitted).

# Appendix 2: Risk assessment of the use of cryopreserved ovarian tissue to restore ovarian function

---

ACART has applied its risk acceptability framework<sup>12</sup> to help it assess the known risks to health in the use of cryopreserved ovarian tissue to restore ovarian function. The framework sets out a process and considerations for ACART to take into account in its analysis of whether or not the risks fall within a level that is acceptable in New Zealand.

Below ACART sets out its comparison of the risks associated with the use of cryopreserved ovarian tissue to restore ovarian function, the principles of the HART Act 2004 applied, and the questions used in its analysis of whether the level of risk is acceptable. Note the consequences table is simply a tool for presenting and comparing information. ACART's analysis is set out in section 4 of this document.

## Risk

'Risk' is a combination of two concepts:

- the likelihood of an effect occurring
- the consequences of an effect if it occurs.

Likelihood and magnitude can be described qualitatively or quantitatively.

## Likelihood

To consider the likelihood of risks associated with the use of cryopreserved ovarian tissue to restore ovarian function, ACART has used the following categories.

	<b>Descriptor</b>	<b>Description</b>
A	Frequent	Is expected to occur again either immediately or within a short period of time (likely to occur most weeks or months)
B	Likely	Will probably occur in most circumstances (several times a year)
C	Possible	Possibly will recur – might occur at some time (may happen every one to two years)
D	Unlikely	Possibly will recur – could occur at some time in two to five years
E	Rare	Unlikely to recur – may occur only in exceptional circumstances (may happen every five to 30 years)

---

<sup>12</sup> <http://acart.health.govt.nz/publications-and-resources/acart-publications/risk-acceptability-framework-used-acart>

## Consequences

To assess the consequences of the risks associated with the use of cryopreserved ovarian tissue to restore ovarian function, ACART has used the following descriptors of consequences.

<b>Descriptor</b>	<b>Descriptions (risks and costs)</b>
Serious	Patients whose death is unrelated to the natural course of the illness and differs from the immediate expected outcome of the patient's management
Major	Patients suffering a major permanent loss of function (sensory, motor, physiological or psychological) unrelated to the natural course of the illness and differing from the expected outcome of patient management
Moderate	Patients with permanent reduction in bodily function (sensory, motor, physiological or psychological) unrelated to the natural course of the illness and differing from the expected outcome of patient management or any of the following: <ul style="list-style-type: none"> <li>• increased length of stay as a result of the incident</li> <li>• surgical intervention required as a result of the incident</li> </ul>
Minor	Patients requiring an increased level of care, including review and evaluation, additional investigations, or referral to another clinician
Minimum	Patients with no injury or increased level of care or length of stay





## Comparing the risks

ACART has used the following table to quantify and compare each aspect of the risk associated with the use of cryopreserved ovarian tissue to restore ovarian function.

Legend:

E = extreme risk	H = high risk	M = moderate risk	L = low risk
------------------	---------------	-------------------	--------------

	Consequences				
Likelihood	Serious	Major	Moderate	Minor	Minimum
<b>A</b> (frequent)	E	E	H	M	M
				<p><b>Surgical risk</b></p> <p>There are no reported increased procedural risks with transplanting thawed ovarian tissue. The procedural risks are considered low risk and no major complications from the operation have been reported. There is however a risk with any surgical procedure, for example, infection, bleeding or damage to adjacent organs.</p>	
<b>B</b> (likely)	E	E	H	M	L
			<p><b>Risk of damage to the tissue</b></p> <p>No reports suggest the cryopreservation procedure imposes any change in the tissue and could lead to unwanted side effects upon transplantation. There are no reports on any potential risks to a woman's health through the use of cryopreserved ovarian tissue in terms of oocytes and subsequent embryos being more abnormal or less viable as compared to mature oocytes obtained in IVF treatment.</p> <p>There is a risk of damage to oocytes and follicles in the ovarian tissue during the cryopreservation procedure. This in turn has an effect on rates of oocyte retrieval and embryo formation in subsequent IVF treatment.</p>		

	Consequences				
Likelihood	Serious	Major	Moderate	Minor	Minimum
C (possible)	E	H	H <b>Pregnancy-related risks</b> There are no known higher pregnancy-related risks for women with transplanted ovarian tissue. The only risk is that their cancer treatment may have affected their ability to carry a pregnancy, for example damage from irradiation of her pelvis.	M	L
D (unlikely)	E	H <b>Concerns with transplanting tissue excised at the time of disease</b> The safety of grafting ovarian tissue excised at the time when the patient experienced active disease is unresolved. To date, there are no studies or reports to secure that the transplanted tissue does not develop or increase the risk of malignancy later on. There is a potential risk of malignant cells from the original cancer being present in the transplanted tissue. If the tissue was excised at the time of active disease, the transplanted tissue may cause a relapse.	M	L	L
E (rare)	H	M <b>Outcomes for children</b> All babies born so far from transplanted cryopreserved ovarian tissue have been reported healthy. There is no information to suggest children born from cryopreserved ovarian tissue are any different from children conceived naturally There are no long term follow-up studies on the children and their development at present, and it is too early to identify any adverse observations of a child's development or psychological outcomes. There is also no systematic reporting of pregnancy and birth data. There is not enough information regarding resultant chromosomal abnormalities, congenital malformations or epigenetic disorders. Due to the lack of this information, future monitoring of health outcomes for children born from the use of cryopreserved ovarian tissue is required.	M	L	L

# Feedback form

Please provide your contact details below.

Name:	
If this feedback is on behalf of an organisation, please name the organisation:	
Please provide a brief description of the organisation if applicable:	
Address/email:	
Interest in this topic (e.g. user of fertility services, health professional, researcher, member of public):	

Please refer to page v for information about:

- Publication of feedback on ACART's website
- Official Information Act requests – possible release of you feedback
- Official Information Act requests – possible release of you name and contact details

We will acknowledge all feedback.

I **request** that my feedback be withheld in full or part from publication on ACART's website. (If you wish a part to be withheld, please clearly indicate which part.)

I **do not** give permission for my name to be published on ACART's website.

I **do not** give permission for my contact details to be published on ACART's website.

If you consider that your feedback, or your name and contact details (if you are submitting on behalf of an organisation), should be withheld under the Act, please state the reasons here:

---

---

---

---

---

---

---

---

# Questions for response

---

## Question 1

*Refer to sections 3 and 4.*

- (a) Do you agree with ACART's assessment of the known risks and benefits to health associated with the use of cryopreserved ovarian tissue to restore ovarian function?

Yes  No

Please give reasons for your views.

- (b) Are there any risks and/or benefits associated with the use of cryopreserved ovarian tissue to restore ovarian function that ACART has not identified or assessed?

Yes  No

If yes, please list below.

## Question 2

*Refer to section 4.*

- (a) Do you agree with ACART's conclusion that the risks associated with the use of cryopreserved ovarian tissue to restore ovarian function falls within a level that is acceptable in New Zealand?

Yes  No

Please give reasons for your views.

- (b) Please note any other comments below.

### Question 3

*Refer to section 4.*

- (a) Has ACART identified all the relevant areas to monitor the use of cryopreserved ovarian tissue to restore ovarian function?

Yes  No

Please give reasons for your views.

- (b) Are there any other areas ACART should monitor?

Yes  No

Please give reasons for your views.

## Question 4

*Refer to section 5.*

- (a) Has ACART identified all the ethical issues relevant to the use of cryopreserved ovarian tissue to restore ovarian function?

Yes  No

Please give reasons for your views.

- (b) Do you agree with ACART's ethical analysis that there are no significant ethical issues associated with the use of cryopreserved ovarian tissue to restore ovarian function?

Yes  No

Please give reasons for your views.

## Question 5

*Refer to section 6.*

- (a) Do you agree that the use of cryopreserved ovarian tissue to restore ovarian function should become an established procedure?

Yes  No

Please give reasons for your views.

- (b) Please note any other comments below.

## Question 6

*Refer to section 6.*

Do you agree with ACART's position that the scope for the use of cryopreserved ovarian tissue to restore ovarian function be limited to the woman from whom the tissue was excised, for her own treatment?

Yes  No

Please give reasons for your views.



## Question 7

*Refer to section 6.*

Do you have any further comments to share with ACART?