Report on the current status of the use of cryopreserved ovarian tissue for the Advisory Committee on Assisted Reproductive Technology (ACART) of New Zealand

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Introductory Comments

This report aims to provide a summary of the current status of the use of cryopreserved ovarian tissue in both humans and experimental animals with a view of informing the Advisory Committee on Assisted Reproduction Technology of New Zealand on the feasibility of undertaking this procedure in New Zealand in the near future.

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 a result, much of the information presented relates to the cryopreservation of ovarian tissue, with a smaller proportion relating to its subsequent use. This reflects the novelty and rapid development of the field. In some important areas there is no experience of use of cryopreserved tissue, for example in children with cancer, yet tissue storage in such individuals is a key aspect for the future.

Female fertility preservation is an area of significant clinical activity in many countries of the world. The main stimulus to this is the dramatic change in survival from a range of malignant diseases eg (Early Breast Cancer Trialists' Collaborative Group, 2005), most notably in childhood cancers (Steliarova-Foucher et al., 2004, Magnani et al., 2006). Overall 80% of children now survive their cancer. In both adult and paediatric oncology practice there has therefore been a shift from survival to quality of life after treatment, and thus the growing prominence of long-term adverse effects. Prominent among these is loss of fertility in both men and women: that female fertility is adversely affected by chemotherapy (including in children) has long been recognized (Warne et al., 1973, Himelstein-Braw et al., 1978, Whitehead et al., 1983, Byrne et al., 1992, Bines et al., 1996).

The field is however characterised by clinical practice determining progress rather than basic research, although the field as a whole is based upon animal research carried out in the early 1990s. It is believed that the first ovarian tissue cryopreservation for fertility preservation in a cancer patient was carried out in Edinburgh in 1993 (Anderson et al., 2008) although the possibility of ovarian transplantation was first pioneered in the 19th century (Gosden, 2008). The first baby

born as a result of this procedure was not, however, until 2004 (Donnez et al., 2004) and at the time of writing there are published reports on less than a dozen babies who have been born through this technique. There is therefore extremely limited information as to the safety and efficacy of this procedure in humans and no information on the long-term outcome of the children born as a result of it. The report below addresses the questions posed in the template provided to the extent that information is available.

A. Current status of procedure/treatment

1. Indicate if the use of cryopreserved ovarian has been "approved" for human use in other countries. Or alternatively indicate that the use of cryopreserved ovarian tissue has not been banned and is being used for reproductive purposes in other countries.

Ovarian tissue cryopreservation is undertaken in many countries in the world. It is however unclear from the published literature how many countries this is carried out in as it is undoubtedly the case that is carried out in countries from which there are no published reports. The majority of the published literature arises from the United Kingdom, Belgium, Denmark, Spain, Israel and the United States. It is clear however, that there is significant clinical activity in numerous other countries including France, Norway, Australia, The Netherlands and Germany with some evidence of activity in New Zealand (Heath and Stern, 2006). Clinical policy documents on this procedure have been published by National and International Societies and Professional Bodies including the American Society for Reproductive Medicine (ASRM), FIGO, The American Society of Clinical Oncology, The American College of Obstetrics and Gynaecology, the British Fertility Society and the joint UK Royal Colleges (British Fertility Society, 2003, Lee et al., 2006, Nakayama and Ueno, 2006, Report of a Working Party of the Royal College of Physicians Royal College of Radiologists and Royal College of Obstetricians and Gynaecologists, 2007, ACOG Committee Opinion No. 405, 2008, Heineman et al., 2008, Practice Committee of American Society for Reproductive Medicine, 2008).

The regulatory aspects of this vary from country to country and are likely to have had a significant impact on clinical practice. In the United Kingdom there was widespread activity in the early years of the century with many ovarian tissues samples being stored in IVF Units. Following the introduction of the Human Tissue Act in 2004 this activity largely ceased with only a very small number of centres being able to continue to operate under those regulations. This was because the standards under which IVF units operate do not meet those required by the HTA, for example in air quality, although both this and many other quality management standards in IVF units have improved considerably in recent years. The regulatory environment has again changed in the United Kingdom such that storage of ovarian tissue is now under the remit of the Human Fertilisation and Embryology Authority (HFEA) following amendment of the HFEAct (1990). This now defines gametes as cells of the germ lineage at any stage of development whereas the previous definition was in keeping with the biological one of a cell capable of fertilisation or being fertilised, and storage or treatment involving gametes requires a license from the HFEA.

There do not appear to be any countries in the world in which ovarian cryopreservation is banned.

2. Identify possible 'uses' of cryopreserved ovarian tissue noting specific situations where this procedure has been used e.g. medical conditions, social reasons etc.

The most common indication for ovarian tissue cryopreservation is in women facing the loss of their fertility from cancer treatment ie chemotherapy, radiotherapy or less frequently surgery. There is, however, a clear indication in women who will be treated with chemotherapy including alkylating agents for non malignant conditions such as autoimmune conditions including systemic lupus erythematosis (Anderson et al., 2008, Elizur et al., 2008) and also in non-malignant haematological conditions such as sickle cell disease (Donnez et al., 2006), which is increasingly treated with bone marrow transplantation.

A further indication is in young women who are likely to go through an early menopause as a result of other medical conditions. The most frequently discussed

diagnosis in this context is Turner's Syndrome and there are reports on the use of ovarian tissue cryopreservation in such women although there remain significant uncertainties over oocyte developmental potential (Hreinsson et al., 2002, Huang et al., 2008, Borgstrom et al., 2009, Lau et al., 2009, Balen et al., 2010). Silber has also reported on a remarkable series of twins discordant for premature ovarian failure, who have undergone transplantation of fresh or cryopreserved ovarian tissue from one to the other, followed by successful pregnancy and delivery (Silber et al., 2008, Silber et al., 2010)

There is the additional possibility of using this approach to preserve fertility for social reasons. The need for significant surgical intervention ie a laparoscopy to obtain ovarian tissue makes this a more invasive procedure than many women or their medical attendance would be prepared to consider. Thus while there is has been debate over the use of oocyte storage particularly involving vitrification for social fertility preservation (Cutting et al., 2009) there are no publications on the use of ovarian tissue cryopreservation for this indication.

3. If it has been approved (or is in use), specify:
   (a) which countries
   (b) when approval was given/use began
   (c) the extent or conditions of the approval/use.

Countries in which ovarian tissue cryopreservation has been reported include the United Kingdom, Copenhagen, Norway, Germany, Belgium, France, Spain, United States, The Netherlands and Israel. It is likely that there are many other countries in which it is also undertaken but from which there have been no published reports. The first reported use was in 1993 in the United Kingdom (Anderson et al., 2008). The various reports from the countries mentioned above do not mention in the published literature any comment on the extent or conditions of approval by their regulatory authority.

In Denmark government approval prohibits the transplant of ovarian tissue from one woman to another (CY Andersen, personal communication) whereas this is not the case in the United States (Silber et al., 2008).

4. If it has been banned (or has proven to be controversial), specify
   (a) which countries
   (b) why it was banned/proved controversial.

There are no reported countries in which this has been banned.

5. Indicate the number of individuals who have used cryopreserved ovarian tissue and/or the number of individuals studied who have used cryopreserved ovarian tissue.

A major deficiency in the field is any sort of register of the number of women who have undergone ovarian tissue cryopreservation or its use. Some reports do, however, describe the experience of individual centres, including from the United Kingdom (Anderson et al., 2008), Denmark (Rosendahl et al., 2008), Israel (Meirow et al., 2007), Spain (Sanchez et al., 2008), the US (Kim et al., 2009), Belgium (Demeestere et al., 2003, Jadoul et al., 2010), France (Poirot et al., 2007) and The Netherlands (Jenninga et al., 2008). The most widespread use of this procedure appears to be in Denmark where a national service has been organised resulting in ready availability of this technique in that country with approximately 350 women now having had ovarian tissue cryopreserved (Schmidt et al., 2010). The number of women who have had cryopreserved ovarian tissue reimplanted is also unclear, but may now be approximately 40 (von Wolff et al., 2009).

6. Describe the information that is available on the outcomes of using cryopreserved ovarian tissue.

Recovery of limited ovarian activity following orthotopic transplantation of cryopreserved ovarian tissue in a cancer patient was first reported in 2001 (Radford et al., 2001), in a woman with Hodgkin's lymphoma who had received significant amounts of chemotherapy before ovarian tissue harvest at the age of 36. Some evidence of short-lived ovarian activity but not ovulation was apparent 7 months after transplantation of the tissue. Subsequent reports have indicated rather shorter timescales for recovery of follicular activity, generally in 4-6 months, which may

reflect the very limited number of follicles likely to have been present in this initial case compared to subsequent experience, which has generally been in younger women with ovarian tissue recovery prior to chemotherapy. A second report demonstrated that orthotopic transplantation could also result in follicular activity and recovery of oocytes (Oktay et al., 2001) although subsequent experience of heterotopic transplantation has not resulted in successful pregnancy.

Ovarian activity in cryopreserved, transplanted tissue in response to exogenous gonadotrophin therapy resulting in presumed ovulation was reported in a woman who had undergone oophorectomy for menometrorrhagia (Oktay and Karlikaya, 2000). In a woman with cervical cancer who underwent bilateral oophorectomy, heterotransplantation under the rectus sheath resulted in follicle development and evidence of ovulatory changes with a serum progesterone concentration of 35nmol/l achieved (Kim et al., 2004). Subsequent experience from that group (Kim et al., 2009) in 4 women following heterotopic transplantation of ovarian tissue has found that ovarian activity can last for as long as 41 months. Experience of oocyte recovery and developmental potential remains very limited: following recovery of a total of 6 oocytes, 3 which were at metaphase 1 and matured in vitro, all 4 metaphase II oocytes were successfully fertilised with some early embryo development in all. While these limited data are supportive of further work to optimize heterotopic transplantation, no pregnancies have been achieved and it is possible that there may be additional safety concerns over oocytes matured in this way rather than following orthotopic transplantation.

The first baby born following transplantation of cryopreseved ovarian tissue was in 2004 (Donnez et al., 2004), to a woman with Hodgkin's lymphoma. Currently, a total of 9 babies have been born from this technique in women with cancer as reported in the world literature. The majority have been born following orthotopic reimplantation of ovarian tissue into the pelvis followed by assisted conception, with a minority born following spontaneous conception (Donnez et al., 2004, Demeestere et al., 2007, Meirow et al., 2007, Andersen et al., 2008, Silber et al., 2008, Sanchez-Serrano et al., 2010) including two woman who have had two children (Demeestere et al., 2010, Ernst et al., 2010). Notably none have been born following heterotopic transplantation of ovarian tissue and assisted conception although embryo

development (Oktay et al., 2004) and a biochemical pregnancy (Rosendahl et al., 2006) have been achieved. Table 1 summaries the human pregnancies that have been described following transplantation of cryopreserved ovarian tissue in cancer patients (as of June 2010).

Ovarian activity with ovulation but not pregnancy has been reported in a 21 year old woman with sickle cell disease in whom ovarian tissue was recovered prior to chemotherapy and bone marrow transplantation, and transplanted 5 years later both to the remaining ovary and in a peritoneal window close to the ovary (Donnez et al., 2006). Recently a successful pregnancy has also been reported in a similar case (Roux et al., 2010).

Pregnancies have also been reported in a remarkable series of monozygotic twins where one had developed premature ovarian failure and ovarian tissue or a whole ovary was transplanted from the sister (Silber et al., 2008, Silber et al., 2010).

Ovarian tissue is generally cryopreserved using a slow protocol with dimethyl sulfoxide (DMSO) or ethylene glycol as cryoprotectant. Advances in oocyte vitrification have led to discussion of this being applied to ovarian tissue with encouraging results in a morphological analysis (Keros et al., 2009). A recent comparison of oocyte recovery from slow cryopreserved versus vitrified human ovary indicated that oocyte survival was comparable in fresh and vitrified tissue, whereas it was reduced by approximately 50% in cryopreserved tissue (Silber et al., 2010). There therefore remain considerable possibilities for technological advances in enhancing the efficiency of this technique.

Table 1

Pregnancies in women with cancer who have had ovarian tissue cryopreserved and later reimplanted

Diagnosis	Age	Surgery	Reimplantation	Pregnancy	Reference
Hodgkin's	25	Unilateral biopsy	Orthotopic	Spontaneous	(Donnez et al., 2004)
Non-Hodgkin's	28	Unilateral biopsy	Orthotopic	IVF	(Meirow et al., 2007)
Hodgkin's	29	Oophorectomy	Ortho and heterotopic	Spontaneous (miscarriage)	(Demeestere et al., 2006)
Hodgkin's	28	Oophorectomy	Ortho and heterotopic	Oocyte from heterotopic, biochem preg	(Rosendahl et al., 2006)
Hodgkin's	24	Oophorectomy	Ortho and heterotopic	Spontaneous*	(Demeestere et al., 2007)
Hodgkin's	25	Oophorectomy	Ortho and heterotopic	IVF, miscarriage	(Andersen et al., 2008)
Hodgkin's	26	Oophorectomy	Orthotopic	IVF*	(Andersen et al., 2008)
Ewing sarcoma	27	Oophorectomy	Orthotopic	IVF	(Andersen et al., 2008)
Breast cancer	36	Unilateral biopsy	Orthotopic	IVF (twins)	(Sanchez-Serrano et al., 2010)

*These two women have subsequently had a second child, conceived spontaneously (Demeestere et al., 2010, Ernst et al., 2010).

7. Describe the information that is available on the risks of using cryopreserved ovarian tissue.

The risk of using cryopreserved ovarian tissue includes surgical risks associated with the procedures and the risk of reimplantation of malignant disease. There are no published data on the surgical risks but these are expected to be as one might expect from standard laparoscopic or mini-laparotomy procedures.

There are no cases of recurrences of malignant disease following reimplantation although this is a subject that has been extensively discussed as it is clear from

animal studies that blood-borne malignant cells can contaminate ovarian tissue and survive cryopreservation and transplantation in an animal model of lymphoma (Shaw et al., 1996). In one report from Israel tissue from a woman with chronic myeloid leukemia that was initially assessed to be not contaminated at the time of storage was subsequently reanalysed and found to be contaminated at the time when reimplantation was being discussed (Meirow et al., 2008). In a further large series of 100 ovarian biopsies from women with breast cancer no instances of contamination were detected (Sanchez-Serrano et al., 2009).

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

It is clear that this technique has the opportunity to preserve fertility to women who would otherwise be sterilised as a result of their cancer therapy (or other medical conditions such as discussed above), where the options are currently very limited. The main potential alternatives are oocyte cryopreservation or vitrification and embryo cryopreservation.

The main advantage of ovarian tissue cryopreservation is that it does not require the involvement of a male partner in fertilizing oocytes and gives a potentially much larger number of germ cells that can be stored than can be achieved with the potential alternative for fertility preservation without fertilisation, i.e. ovarian stimulation and oocyte cryopreservation. Oocyte cryopreservation was first reported to result in successful pregnancy in 1986 (Chen, 1986), but subsequent take-up has been slow reflecting poor survival of oocytes. This is a rapidly developing area of research with significant technical developments that have resulted in improved oocyte survival and fertilization rates, most notably by the increased use of vitrification which seems likely to become the preferred technique with fertilization and development rates comparable to those obtained with fresh oocytes (Cobo et al., 2008, Cobo et al., 2008, Cutting et al., 2009). Improvement in this technology has also benefited from non-medical, legislative developments: legislation in Italy forbids fertilisation of more than 3 oocytes at a time, thus oocyte cryopreservation has been particularly developed in that country (Fadini et al., 2009).

In general, oocyte recovery follows ovarian stimulation using the same protocols as are used in IVF. This results in recovery of mature oocytes (ie at metaphase II), but requires a similar timescale as IVF. Immature oocyte recovery has also been reported without ovarian stimulation and appears to be possible at all stages of the menstrual cycle, with in vitro maturation prior to cryopreservation or vitrification (Demirtas et al., 2008). Immature oocyte recovery can also be combined with ovarian tissue cryopreservation (Huang et al., 2008).

Embryo cryopreservation is an established technology and thus widely available in most IVF Units in the world. Its potential use by women facing chemotherapy is limited by the need for ovarian stimulation and by the need for fertilization of the recovered oocytes. Thus, unless donor sperm is used, the women is committing her oocytes to be fertilized by a partner who will then have joint ownership of the resulting embryos. Subsequent breakdown of the relationship may then prevent the woman from the ability to use those embryos. It is striking that there are extremely limited published data on IVF outcome in women treated as an emergency for malignancies but it is clear that this is widely used around the world and the preferred option for women in a stable relationship where pressure of time does not preclude the opportunity for superovulation and oocyte retrieval. The limited data that are available indicate that normal numbers of oocytes and fertilization rates are achieved in this context, although implantation rates are not available (von Wolff et al., 2009).

A potential issue for women choosing to have ovarian stimulation followed by either oocyte or embryo cryopreservation is the resulting high estrogen production and exposure to their cancer: in contrast, ovarian tissue cryopreservation requires no hormonal treatment. This has been largely discussed in the context of women with estrogen receptor positive breast cancer in whom the exposure to supraphysiological estrogen concentrations, albeit for a short period, may pose a risk. To avoid this, regimens based on the use of the estrogen antagonist tamoxifen or an aromatase inhibitor, alone or with low dose FSH, have been described (Oktay et al., 2003, Oktay et al., 2006). Tamoxifen use antagonises the effect of estrogen but does not reduce its production, whereas aromatase inhibition results in very low estradiol concentrations. Acceptable numbers of oocytes can be recovered using these approaches. A similar protocol has also been used for ovarian stimulation in women

with early endometrial cancer (Azim and Oktay, 2007). The developmental potential of the oocytes cryopreserved after these regimens is unknown, as is whether such regimens confer a real survival benefit to the woman. Data available so far in women with breast cancer are inadequate to support conclusions of either benefit or risk (Azim et al., 2008).

Ovarian tissue cryopreservation also has a particular unique place for young women, particularly prepubertal girls for whom there is no alternative at present as ovarian stimulation would be inappropriate (Schmidt et al., 2010). A series of 49 prepubertal girls who underwent unilateral oophorectomy and ovarian tissue cryopreservation has been described (Poirot et al., 2007), and laparoscopic ovarian biopsy has also been described in young girls (Feigin et al., 2007, Anderson et al., 2008). A recent report describes a series of 58 girls and young women who have undergone ovarian tissue cryopreservation following laparoscopic oophorectomy or cortical biopsy (Jadoul et al., 2010). That comprehensive report reviews the issues regarding this procedure in children, highlighting the lack of evidence or consensus as to the appropriate indications for this procedure and the absence of outcome data in keeping with it's recent development. The potential to offer ovarian tissue cryopreservation in cancer patients in this age group without delaying chemotherapy is also demonstrated.

9. Describe any areas where there is deficient information about the use of cryopreserved ovarian tissue (i.e. potential risks, benefits and outcomes).

As indicated above, there are substantial deficiencies in our knowledge of current practice in the field of ovarian tissue cryopreservation and subsequent use of that tissue. While a number of babies have been born it is unclear how many women have had ovarian tissue replaced. More fundamentally it is unclear as to how best to select the women to whom to offer this treatment, which surgical procedure is optimal (oophorectomy or cortical biopsy), methods for freezing tissue are probably not optimally developed and methods for the replacement of tissue are based on clinical sense rather than experimental evidence.

B. Information from human studies (review if applicable)

10. Outline the efficacy of using cryopreserved ovarian tissue, including:
    (a) fertilisation rates
    (b) survival rate of the oocytes following cryopreservation and then re-implantation
    (c) pregnancy rates (please compare to use of fresh mature eggs in IVF)
    (d) live birth rates (please compare to use of fresh mature eggs in IVF).
    (e) diagnostic accuracy of the procedure.

There are no substantial data available on any of these issues. It is clear, however, that many oocytes are lost during the cryopreservation and transplant process: in sheep this is estimated to be approximately 70% (Baird et al., 1999). Some information can be obtained on fertilisation rates and subsequent implantation rates of oocytes recovered following ovarian transplantation where the women have subsequently undergone a super ovulation and IVF. Andersen *et al* reported an overall fertilisation rate of 44% of oocytes recovered (Andersen et al., 2008). Transfer of a total of 10 embryos obtained where the cryopreserved ovarian tissue had been grafted to the residual ovary in 6 women resulted in 2 live births and one additional clinical pregnancy (Andersen et al., 2008). A recent Spanish report (Sanchez-Serrano et al., 2010) described recovery of 16 mature oocytes of which 78% were fertilized, and twins were born following replacement of 2 embryos. In a further report of 4 women who had IVF following orthotopic transplantation of cryopreserved tissue, 16 oocytes were recovered: 10 were at metaphase II of which 5 fertilised but no pregnancies resulted (Dolmans et al., 2009). It remains unclear therefore whether fertilisation and implantation rates are compromised compared to those normally achieved in IVF.

Data are also available following heterotopic transplantation (Oktay et al., 2004): in this case report a woman had eight oocyte retrievals following transplant of ovarian tissue beneath the skin of the abdomen, and 20 oocytes were retrieved. Eight were suitable for fertilisation but only one fertilised and developed into a four cell embryo. More positive results have been reported (Andersen et al., 2008) but importantly no clinical pregnancies have been achieved. On the basis of this limited evidence it

therefore appears that heterotopic transplantation results in compromised follicular and oocyte maturation.

It would appear from the literature that pregnancy rates are high in women who have had ovarian tissue transplanted but it is unclear how much this reflects selection bias in the literature. Thus, for example, in the report by Andersen *et al* in 2008 four of six women conceived following assisted reproduction although two had an early miscarriage (Andersen et al., 2008). This should be interpreted in the light of the significant spontaneous pregnancy rate in those women who did not have cryopreserved ovarian tissue replaced. One of these two women who went onto have a baby has now been reported to have had a second successful spontaneous conception and pregnancy and delivery (Ernst et al., 2010).

11. Detail any risks to health through the use of cryopreserved ovarian tissue, including (but not limited to):
    (a) any potential side effects (please compare to use of fresh mature eggs in IVF)
    (b) health outcomes for female patients. This includes both short term and long term (e.g. the treatment could increase the risk of cancer many years later)
    (c) any suggested exclusions of potential patients based on clinical indicators (e.g. cancer, diabetes)
    (d) health outcomes for male patients
    (e) Observed damage to the oocytes.

Risks to health include from the surgery required to retrieve the tissue. This is normally at laparoscopy and thus is a separate procedure for women to undergo. It may however on occasion be combined with another procedure such at the insertion of lines or on occasion at the time of Caesarean section (Anderson et al., 2008). There are, therefore, risks associated with this procedure. Further surgical risks are entailed at the time of transplant of the tissue, again generally at laparoscopy. In women who subsequently have conceived spontaneously there are no addition side effects. Other women have undergone assisted conception and therefore have been exposed to the same risks as women undergoing IVF normally although one would

anticipate the risk of ovarian hyperstimulation to be very low because of the severely reduced ovarian reserve.

A further health risk would be from the delay of initiation of treatment of their cancer at the time of diagnosis. This will often be minimal as no pretreatment is required prior to ovarian cryopreservation. This is in contrast to women having IVF prior to initiation of chemotherapy where significant delays are generally incurred.

There is no indication that this treatment increases the risk of subsequent cancer in these patients other than through the potential reimplantation of the disease via the transplanted tissue. There have, however, been no cases of this reported. A thorough survey of 100 ovarian biopsies taken from women with breast cancer showed no evidence of ovarian contamination (Sanchez-Serrano et al., 2009) although the ovary is a recognised site of metastatic spread. See also comments in section 7 above.

Inclusion and exclusion criteria for patients having this therapy have been published by only 2 groups (Anderson et al., 2008, Schmidt et al., 2010). Patients may, however, be felt not to be suitable for laparoscopy at the time of diagnosis and this would frequently be the case for example in acute leukemia. The most common diagnoses in women who have had ovarian tissue cryopreserved are lymphoma and sarcoma, and women with these conditions will generally be expected to be fit for laparoscopy assuming they do not have any other concurrent medical problems.

There are no issues for male patients.

Many oocytes will be lost during recovery freezing and transplantation as described above. It also appears that heterotopic ovarian transplantation is associated with abnormal follicular and oocyte maturation (Oktay et al., 2004). The fertilisation rates of oocytes recovered following orthotropic transplantation by contrast appears comparable to that following recovery from normal ovarian tissue (Andersen et al., 2008) although a second report has indicated that oocyte developmental potential following ovarian tissue cryopreservation/orthotopic transplantation and IVF was low (Dolmans et al., 2009).

11. Detail the obstetric outcomes (risks and/or benefits to health) where applicable, including (but not limited to):
    (a) observed damage to the oocyte or embryo
    (b) neonatal/infant complications
    (c) chromosomal abnormality
    (d) congenital malformations (i.e. birth defects)
    (e) child development (physical, psychomotor and cognitive)
    (f) psychological outcomes for child and family
    (g) epigenetic disorders (i.e. imprinting)
    (h) maternal outcomes (including complications).

There are very limited data available on obstetric complications because of the very limited number of pregnancies that have occurred in women following ovarian transplantation. Other than early miscarriage, no pregnancy complications have been reported, and the infants born have been reported to be normal and healthy and of normal weight at birth. No further information is available on their subsequent development thus it is unclear as to whether they might be at risk of subsequent developmental, psychological or epigenetic disorders. Thus at present the results seem very encouraging in terms of normal pregnancy and delivery of a healthy infant but no certainty can be applied to this in the light of the extremely small numbers involved. Similarly no unusual maternal complications have been reported: in the two original Danish pregnancies one had mild pre eclampsia and was delivered by Caesarean section at 37 weeks, the second had a normal pregnancy and delivery at 39 weeks (Andersen et al., 2008).

12. Indicate if the use of cryopreserved ovarian 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:
    (a) toxicity
    (b) interactions
    (c) long term effects of medications.

No medical therapy is involved in ovarian tissue recovery, cryopreservation or transplantation. All agents used during the cryopreservation process are in widespread use in organ and tissue transplantation. Where assisted conception is used following transplant of the tissue back into the woman standard IVF procedures and drugs are used.

It is possible that a woman might incur a delay in initiating her cancer therapy as a result of having ovarian tissue stored. No pretreatment prior to laparoscopy is required and thus it can generally be carried out at short notice with minimal if any delay. As mentioned above this contrasts with the significant delay often incurred if a women undergoes superovulation and IVF prior to initiating cancer therapy.

13. Indicate if other treatment (e.g. for cancer) might be delayed as a result of using cryopreserved ovarian tissue.

It is possible that other treatment might be delayed by having ovarian tissue cryopreserved. This might reflect the need for additional consultations, and the surgery itself. It is likely however that this will be minimal or can be avoided altogether with appropriate service organization, particularly with the development of appropriate channels of communication between oncologists and reproductive medicine specialists.

There are no reasons to consider that the use of cryopreserved ovarian tissue will interfere with other treatments. Replacement of ovarian tissue is an elective procedure that would only normally be undertaken where the woman is in good health with no evidence of residual cancer that requires further treatment.

14. Indicate whether any associated risks may occur as part of the surgical intervention carried out in the use of this procedure.

Additional surgical risks result from the fact of having a surgical procedure to recover the tissue and a further one to replace it. In general these risks will be similar to a laparoscopy under normal circumstances for gynecological indications. There may be additional risks based upon an individual patient’s circumstances such as

neutropenia or thrombocytopenia but there have been no reports in the literature of women having surgical complications as a result of these procedures.

15. Indicate the potential age range of this treatment.

This procedure is theoretically applicable to women and children of any age prior to the menopause. It has been argued that as this is an experimental procedure it should only be applied to relatively young women ie less than 30 but others have suggested that an upper limit of 35 at least is appropriate (Anderson et al., 2008, Schmidt et al., 2010). This may be better determined by assessment of a woman's reproductive biological age rather than chronological age eg by assessment of the ovarian reserve. These arguments are based on the natural decline in follicle number with age and the fact that many follicles will be lost during the cryopreservation and transplant process. The successful pregnancies that have occurred have been in women in their late 20s and early 30s (see table 1 above). However, because the tissue could potentially be stored for many years it is theoretically possible to reimplant the tissue in women at any subsequent age.

There is no minimum age at which this procedure can be carried out. There are reports in the literature of this being carried out on young children, and it appears that it has also been considered in children as young as 18 months old (Andersen CY personal communication). Clearly this raises significant ethical considerations which have been widely discussed (Wallace and Walker, 2001, Zoloth et al., 2008, Schmidt et al., 2010).

16. Indicate if the use of cryopreserved ovarian tissue can increase the risk of other disease (e.g. cancer).

There is no indication that the use of cryopreserved ovarian tissue increases the risk of subsequent ovarian or other disease. Risks associated with reimplantation of the original malignancy are discussed above.

Risk of cancer in the offspring of cancer patients has been addressed in large retrospective studies. These have not shown any evidence of increased cancer risk

in the offspring of long term cancer survivors with the exception of inherited cancers such as retinoblastoma (Mulvihill et al., 1987, Sankila et al., 1998).

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

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

18. Specify the number of animals studied who have used cryopreserved ovarian tissue for reproduction.

19. Outline the efficacy of using cryopreserved ovarian tissue:
    (a) fertilisation rates
    (b) survival rate of the oocytes following re-implantation of ovarian tissue
    (c) embryo development rates
    (d) pregnancy rates (please compare to use of fresh mature eggs)
    (e) live birth rates (please compare to use of fresh mature eggs)
    (f) diagnostic accuracy of the procedure.

20. Detail any risks to health of using cryopreserved ovarian tissue, including (but not limited to):
    (a) any potential side effects
    (b) health outcomes for male subject
    (c) ongoing development of offspring born as a result of the procedure.

21. Detail the obstetric outcomes (risks and/or benefits to health), including (but not limited to):
    (a) neonatal/infant complications
    (b) chromosomal abnormality
    (c) congenital malformations (i.e. birth defects)
    (d) offspring development (physical, psychomotor and cognitive)
    (f) epigenetic disorders (i.e. imprinting).]

18-21. Questions have been combined for clarity in the light of the modest amount of data available.

The use of cryopreserved ovarian tissues has been investigated in a number of animal species. These include mouse, rat, rabbit, pig, goat, cow and importantly sheep. The latter has been extensively investigated as a mono-ovulatory large mammalian species with many reproductive characteristics in common with the human. The data available will be presented by species but, in general, is not sufficiently exhaustive to give reliable information as to oocyte developmental competence and pregnancy rates, and health of offspring. Where available the limited data will be presented.

Ovarian tissue cryopreservation is well established in mouse following the confirmation of early reports (Carroll and Gosden, 1993). Subsequent work confirmed normal litter size and normal reproductive lifespan in that species (Candy et al., 2000), with no evidence of congenital abnormalities in the pups of the 10 animals which received frozen ovarian grafts. Importantly the possibility of epigenetic abnormalities resulting from cryopreservation have been addressed in the mouse in a recent report (Sauvat et al., 2008), a subject which has not been addressed in other species. This study showed no change in epigenetic marks in the offspring but they did report substantial loss of follicle number in contrast to earlier reports indicating conservation of reproductive life span. Epigenetic abnormalities have been a cause for concern in association with human assisted conception (Gosden et al., 2003, Cutfield et al., 2007, Manipalviratn et al., 2009) thus these data are reassuring if they can be extrapolated to the human.

There are a small number of reports of ovarian tissue cryopreservation applied to rat and rabbit. In the rat this has been proposed as a method for gamete banking. 25% of animals receiving ovarian tissue transplants were reported to become pregnant, each bearing two to three litters ie indicative of a reduced reproductive life span. In the rabbit transplant of cryopreserved ovarian tissue following oophorectomy resulted in pregnancy in all of five rabbits with no reports of abnormality in the offspring (Almodin et al., 2004). In both species successful transplantation of whole ovary with microvascular reanastomosis has been reported, although with considerable loss of

follicle number (Wang et al., 2002, Chen et al., 2006). In the rat, 4 of seven transplants were ovulatory and 1 successful pregnancy was achieved (Yin et al., 2003). In the rabbit, 10 of 12 animals showed evidence of ovulation (Chen et al., 2006). Data are available on oocyte maturation in the rabbit (unlike many other species) with the demonstration of very similar oocyte maturation and blastocyst formation in animals receiving cryopreserved ovarian tissue transplants compared to untreated controls and those receiving fresh transplants (total of 25 animals in the study) (Chao et al., 2008).

Ovarian tissue cryopreservation has been reported in both cow and goat. In the latter follicular development was documented in animals receiving cryopreserved ovarian fragment (10 animals) and three out of five were confirmed to have ovulated although no pregnancies resulted (Santos et al., 2009). In the bovine there are limited reports but vitrification of ovarian tissue has been reported (Kagawa et al., 2009) in six cows. That study reported over 98% oocyte viability but there are few details presented. There are also isolated reports of ovarian tissue cryopreservation in the horse and macaque monkey (Kardak et al., 2007, Matsukawa et al., 2007), but useful data are very limited. There are also reports of ovarian transplantation in poultry but this was of fresh rather than cryopreserved tissue (Song and Silversides, 2008). This has been proposed but not demonstrated as a method for fertility preservation of rare avian species.

A considerable body of work has derived from investigation of ovarian tissue and whole ovary transplantation in the sheep. Indeed, this whole field was largely initiated following pioneering work by Gosden *et al* in that species with demonstration of ovarian activity and spontaneous pregnancy following transplantation of cryopreserved ovarian tissue (Gosden et al., 1994). Subsequent data from that group demonstrated that transplanted cryopreserved tissue could result in ongoing ovarian activity for two years although the number of follicles present in the ovary was drastically reduced (Baird et al., 1999). These experiments involved eight ewes. This study did, however, demonstrate that the majority of follicle loss occurred as a result of engraftment rather than the cryopreservation process. Subsequent data on the sheep indicated that approximately 70% of primordial follicles are lost during this combination of processes (Demirci et al., 2001).

Subsequently work has explored the use of different cryoprotectants (Tsuribe et al., 2009), and in vitro follicle culture following cryopreservation (Muruvi et al., 2009). Further work has been reported on follicle and oocyte survival. In a recent report (Massardier et al., 2010) it was reported that in six animals per group freezing did not affect the number of primordial follicles but post-graft survival was only 6.3% of primordial follicles in cryopreserved tissue compared to 5.1% in fresh transplanted tissue. Comparable data have been obtained following cryopreservation and transplantation of whole sheep ovary using microvascular techniques after initial studies confirmed follicle survival, and subsequent growth and hormone production with the possibility of recovery of developmentally competent oocytes (Bedaiwy et al., 2003, Arav et al., 2010). The potential for live birth was confirmed in a study of 9 ewes; 4 showed evidence of ovulation after reimplantation using microvascular reanastomosis and 1 conceived spontaneously and deliverer a healthy lamb (Imhof et al., 2006). Histological analysis suggests a loss of primordial follicle number of over 90% in transplanted tissue but this was similar in cryopreserved and fresh ovary (Onions et al., 2009). A further study has followed sheep up for six years following whole ovarian transplantation at the end of which two animals were confirmed to be still ovulating and a very small number of oocytes were obtained from these ovaries at the germinal vesicle stage and matured to metaphase II (Arav et al., 2010). Heterotopic transplantation has also been investigated in the sheep (Grazul-Bilska et al., 2008) with the demonstration of some limited follicular growth but no evidence of ovulation.

The sum of animal data provides essentially proof of concept that ovarian tissue can be cryopreserved, and orthotopically reimplanted tissue resulted in the potential to achieve spontaneous pregnancy. The most substantive data demonstrate that in sheep, rat and mouse cryopreservation and transplantation is associated with considerable loss of primordial follicles, generally in the order of 60-80%, and that this therefore results in a considerably shortened reproductive lifespan compared to the natural state. The very small number of live births following this procedure in animal species does not contain any report of abnormalities during pregnancy of the offspring and as mentioned above there are some limited data analysing epigenetic

imprinting in the mouse only. These data are, therefore, insufficient to confirm the safety of the use of cryopreserved ovarian tissue with confidence.

D. Feasibility of undertaking the Procedure in New Zealand

22. Comment on the feasibility of this procedure being undertaken in New Zealand i.e. the requirement for any specific clinical capability and other associated medical processes.

The requirements for this procedure include the ability to perform ovarian cortical recovery or oophorectomy, a facility for cryostorage of the tissue and resources for subsequent reimplantation. Quality control measures are also required. In general these facilities are widely available in IVF units and associated hospitals thus the appropriate model will be determined by the relative availability of oncology and gynaecology services for referral and ovarian surgery, and of facilities such as IVF units offering cryopreservation. The Danish model may be appropriate. This is a hub and spoke model where the woman undergoes counseling, assessment and unilateral oophorectomy in a regional centre and the tissue is transplanted to a central facility for processing and storage. It appears that ovarian tissue can be transported for several hours prior to cryopreservation without excessive damage: an interval of 5 hours appears to be tolerable (Andersen et al., 2008) although no quantitative analysis of follicle loss with time has been performed.

This model however depends upon the local regulations regarding human tissue transplantation and thus for example would be much more difficult to instigate in the United Kingdom. In the United Kingdom the licensing requirements of the Human Fertilisation and Embryology Authority require all aspects of the procedure to be closely governed thus essentially the surgery can only be performed in the central location close to the tissue bank. This therefore requires the woman to travel for the whole procedure, rather than her ovary travelling following removal: the large number of IVF units in much of England means that this can be achieved fairly readily.

In a variation of this all procedures are carried out in one centre. This has been the model developed in Edinburgh, based around the presence of a national Tissue Bank facility providing expertise in tissue storage and associated regulatory aspects. This requires the patient to travel, which in practice has not proved problematic although may raise funding issues. Some women have traveled over 300 miles the day before the procedure, and returned the day after. This limits the potential for face to face counseling prior to the procedure, which has been addressed by discussion with the treating oncologist and telephone discussion with the patient prior to travel.

The surgical skills required are generally those of any gynecological surgeon carrying out laparoscopic surgery. Paediatric laparoscopic surgical skills and involvement of a surgeon with those skills is also required. More critical is the need for excellent channels of communication between oncology and the reproductive medicine team, and with the tissue storage facility. The nature of the process requires all consultations, the surgical procedure and the opportunity for tissue storage to be carried out at short notice in the great majority of women and children for whom this is appropriate ie those with cancer, although there may be less time pressure in those with non-malignant conditions. In general, European reproductive medicine centres which offer this appear to have developed systems with dedicated staff members who liaise with the relevant parties and ensure good communication and rapid access to the procedures involved. Clearly all aspects of this have funding implications.

While the surgical skills required for oophorectomy or ovarian biopsy are widely held, and similarly reimplantation of ovarian tissue is uncomplicated, this does not apply to prospects for whole ovary transplantation. Some very early experimental success with whole human ovary cryopreservation has been reported (Bedaiwy et al., 2006) but the data from sheep in particular described above indicate the laboratory and surgical challenges that need to be overcome to optimise this procedure.

A survey of Paediatric Oncology Units in Australia and New Zealand investigated policy and practice for fertility preservation in children and adolescents in those countries (Heath and Stern, 2006). Data from two centres in New Zealand were included. This survey indicated that some centres were offering fertility preservation techniques (including ovarian tissue cryostorage and treatment with GnRH analogues) but it is unclear whether this aspect included the 2 New Zealand centres.

23. Outline any health issues or procedural risks identified in countries where this procedure has being approved and undertaken.

There is no significant published literature specific to these points. No negative health issues have been identified in relation to cryopreservation of ovarian tissue, with the exception of issues discussed elsewhere, ie immediate risk of the surgical procedure, possibility of delaying treatment for serious medical conditions, and theoretical risk of reimplanting malignant disease.

Procedural risks related to the processes involved include errors at several stages of the process. Those relatively specific to this procedure rather than clinical activities in general include delays in communication resulting in non-availability of the procedure, bacteriological contamination of the ovarian tissue between surgical retrieval and storage, contamination during storage, and laboratory error during cryopreservation resulting in tissue damage. This would include excessive delay in transporting ovarian tissue where that is undertaken (eg in the Danish model). Other aspects may relate to reimplantation of the tissue at a later date.

Tissue storage is generally a highly regulated process which reduces the chances of errors such as these. Patients are screened for infectious disease (generally Hepatitis B and C, HIV) according to local regulations and population characteristics, bearing in mind that there may be the potential for inter-specimen transcontamination during storage (reduced by storage in the vapour phase of liquid nitrogen rather than in the liquid phase), but that tissue samples are ultimately destined for reimplantation in the original patient rather than

donated for the treatment of another. The latter has however been described (Silber et al., 2008).

E. General

24. Specify any alternative procedures or treatments that could be used to gain the same result (i.e. preserve fertility). If so please detail.

Other options for fertility preservation in women include IVF with embryo cryopreservation and oocyte cryopreservation (also discussed in Section 8 above). Women may choose to undergo a normal IVF procedure prior to starting chemotherapy with mature oocytes aspirated following superovulation, fertilised by the patient's partners sperm (or if none available potentially using donated sperm) and the subsequent early embryos cryopreserved as in routine IVF practice. This is a well established procedure potentially offered by all IVF Units. The main disadvantages are that there is significant time involved in this, the woman undergoes the normal risks associated with IVF including hyperstimulation, and there is the requirement for the eggs to be fertilised which will involve the commitment of a male partner in most circumstances. Subsequent use of those embryos may require the continued consent of the male partner as well as the female.

Oocyte cryopreservation is a rapidly evolving technology particularly with the more widespread introduction of vitrification rather than conventional cryopreservation. In general oocytes are cryopreserved/vitrified after superovulation ie at the metaphase II stage. There are, however, reports of oocyte vitrification following recovery of immature oocytes which are then matured in vitro (Demirtas et al., 2008). Successful pregnancy has been reported following this procedure (Chian et al., 2009) but information is very limited. It is additionally likely that in many cases oocyte cryopreservation either following superovulation or aspiration of immature follicles will result in a very limited number of oocytes being available for future fertility which may significantly limit the opportunity for pregnancy.

A further method used with the objective of fertility preservation is ovarian transposition (oophoropexy). This involves surgically moving the ovaries out of the

field of radiation and is applicable to women who will be receiving pelvic irradiation eg for cervical cancer. This does not, however, necessarily provide complete protection for the ovary as while it may be out of the direct beam there may be a significant scatter dose. In addition, the blood supply to the ovary may be compromised resulting in a risk of ovarian failure. There are only observational reports on this procedure and a recent review indicated that between 50% and 90% of women who have pelvic irradiation after oophoropexy still suffered early ovarian failure (Wo and Viswanathan, 2009). An additional disadvantage of this procedure is that by moving the ovaries out of the pelvis it may render them inaccessible for both spontaneous fertility and assisted reproduction. In conclusion, therefore, this procedure is not based on evidence from randomised controlled trials and may introduce later difficulties for fertility.

An alternative approach to fertility preservation in women is by pharmacological protection using a gonadotrophin releasing hormone analogue or the combined oral contraceptive pill. Both are in widespread clinical use but for neither is there convincing evidence of efficacy (Beck-Fruchter et al., 2008, Blumenfeld and von Wolff, 2008) as most studies have been poorly or uncontrolled, primarily from retrospective case control studies. The biological basis for the proposed protection is unclear, but it is possible that the reduced ovarian blood flow secondary to a reduced number/size of growing follicles might reduce the dose of chemotherapy to the ovary.

An early randomised control trial failed to show any benefit but was very underpowered (Waxman et al., 1987). A more recent study in Egypt involved randomisation of 80 women aged less than 40 with breast cancer who were treated with either a GnRH agonist or chemotherapy alone (Badawy et al., 2009). The results appear very promising with 89% of women in the study group resuming menses with evidence of ovulation in 69%, compared to 33% and 26% respectively in the control group. While this appears very promising the data from the control group are rather surprising as one would normally expect a substantially smaller effect on ovarian function in that age group from the chemotherapy regimes used and indeed the results obtained in the GnRH agonist treated group are more in line with results expected in a young chemotherapy treated population such as that studied.

A recent randomised controlled trial of the effect of GnRH agonist or oral contraceptive pill treatment as protection against ovarian toxicity in Hodgkin lymphoma was stopped after interim analysis of only 23 patients because of evidence of absence of benefit (Behringer et al., 2010). Further large studies are underway (Leonard et al., 2010). Whilst this approach would not be appropriate in prepubertal girls it would potentially be appropriate in adolescents and one study has addressed this (Pereyra Pacheco et al., 2001). In this small study nine young women aged between 15 and 20 years old underwent chemotherapy for bone marrow transplantation: all five who received a GnRH agonist resumed menses after treatment whereas all four who did not receive this treatment developed amenorrhea.

An alternative approach only thus far reported in animal studies is the use of a chemoprotective agent. Two approaches that have been found to provide protection against radiotherapy and cyclophosphamide chemotherapy respectively are the administration of sphingosine-1-phosphate (Morita et al., 2000) and by inhibition of the c-Abl-TAp63 pathway by administration of the monoclonal antibody imatinib (Gonfloni et al., 2009). While there are at present no data relating to the ovary, a further agent whose mechanism of action is as an immunomodulator has also been reported to reduce the spermatogenic damage caused by cyclophosphamide administration in mice (Carmely et al., 2009).

25. List any alternative methods/treatments to the use of cryopreserved ovarian tissue and;

(a) Discuss how the benefits to health of the alternative procedures/treatments compare to the benefits of using cryopreserved ovarian tissue.

(b) Discuss how the risks to health of the alternative procedures/treatments compare to the risks of using cryopreserved ovarian tissue.

These issues are discussed in Sections 8 and 24 above.

26. Specify and detail any additional information related to the risks or benefits to health of using cryopreserved ovarian 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 eggs) that have previously undergone cryopreservation.

An additional potential benefit to health of using cryopreserved ovarian tissue will be from the non-reproductive benefits of ovarian function. These most obviously include protection from osteoporosis and there may also be benefits to cardiovascular health. Additional short-term benefits may be the prevention of the other side effects of estrogen deficiency such as hot flushes.

27. Outline any long-term follow-up studies presented to date and any planned for the future.

The field as a whole suffers from the absence of long term follow up data. A most important piece of missing data is a comparison of subsequent fertility in those women who have had ovarian tissue cryopreserved but not replaced. Data that are available indicate the risks to fertility from particular therapies may in some instances be overestimated, as indicated by significant natural fertility rates in women who have had ovarian tissue cryopreserved but not reimplanted (Anderson et al., 2008, Rosendahl et al., 2008). This also raises the possibility that some pregnancies that have occurred in women following ovarian tissue replacement might have arisen from residual, non-cryopreserved tissue. A clear example of this has been described (Bath et al., 2004), and it is common for fertility practitioners to see spontaneous pregnancies in women who have been clearly shown to have had a premature menopause. Conclusive proof of this would require pregnancy following heterotopic transplantation and IVF, but none have been reported. Registries of women undergoing this procedure have been established particularly in Denmark and it is anticipated that these will provide invaluable information in the future to some of these issues but there are as yet no published data. It is unclear, however, whether any long term follow up studies are planned for the children conceived as a result of this procedure: this would also appear to be an imperative.

28. Comment on the quality of the published research.

This field is very much in its infancy as reflected by the fact that many more reviews have been published than children born. In general, the data consist of case reports and small series thus there is the opportunity for significant publication bias in favour of positive outcomes. More comprehensive presentations of overall outcomes are lacking. This severely limits the ability to comment critically on the efficacy and safety of this procedure both for the woman and her potential offspring. Despite this, ovarian tissue cryopreservation appears to hold important promise for girls and young women in particular, for whom the option of embryo cryopreservation is not available. The major alternative technology for such patients is oocyte cryopreservation or vitrification, but current data suggests that this offers more limited scope for future pregnancy because of the small number of oocytes that can be retrieved in a short period of time. The combination of different techniques can also be used appropriately in some cases.

29. List references to all published and peer reviewed research used in the report.

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