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Cryopreservation of female reproductive potential

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A B S T R A C T

Storing female reproductive potential can offer enhanced prospects for future conception in women whose fertility is threatened by cytotoxic therapies. Human female reproductive potential can be cryopreserved and stored at very low temperatures as embryos or gametes. Gamete (oocyte) cryopreservation circumvents potential issues associated with ownership when future use is being considered and may, therefore, be more generally acceptable as an approach. Advances in the technology, in particular the clinical application of vitrification, have significantly improved the outcomes from mature oocyte cryopreservation, which are now comparable to those from embryo cryopreservation. In cases where mature oocyte cryopreservation is not feasible, ovarian cortex containing primordial follicles can be cryopreserved, and over 100 births have now been reported following grafting of stored ovarian tissue. Ovarian tissue cryopreservation is now an established approach to preserve future fertility for young women; however, the efficiency is difficult to determine particularly for the prepubertal tissue with a scarcity of data.

Background

The ability to store biological material at very low temperatures over extended periods of time (cryopreservation) has provided potential solutions in a wide range of biological and clinical situations. Perhaps nowhere is this more evident than in its application in human assisted reproductive technologies (ARTs). In this chapter, the specific role of the cryopreservation of female reproductive potential in current clinical practice is reviewed.

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Who needs the technology?

The most obvious need for the cryopreservation of female reproductive potential, and that which has stimulated much of the research into technologies for the cryopreservation of the female gamete, relates to the rapid onset of loss of fertility that is often observed following cytotoxic therapy for cancer.

Long-term survival rates from various types of cancer have increased over the last decade because of improvements in cytotoxic therapies leading to an increasing population of childhood and adolescent cancer survivors with all the normal life expectations including reproduction. As a consequence of these cytotoxic treatments, the fertile window is either short lived or eradicated in women. The distress caused by the possible loss of fertility for these women has been emphasized in patient surveys [1] and a significant level of regret when fertility has not been discussed in paediatric patients reported [2]. The impact of cytotoxic treatments on fertility is related to chemotherapeutic agent and/or radiation dose and female age, which is generally reflected in the ovarian follicle population [3]. The relative risk of premature ovarian failure associated with various types of chemotherapeutic agents and radiation doses has been reviewed by a number of authors [4], and alkylating agents (e.g. cyclophosphamide) pose the greatest risk. The mechanism of action of the chemotherapeutic agent in the ovary appears to be by a cyclic destruction of growing follicles, which would normally inhibit the recruitment of dormant follicles.

Cytotoxic therapies are also used for a number of non-oncological conditions, such as autoimmune disease. The long duration of treatment using high doses of cyclophosphamide for SLE can have devastating consequences with a high proportion of these young women experiencing premature menopause [5], although the introduction of new drugs and reduced doses have a lesser impact on fertility.

Fertility preservation may also be of benefit in patients with mosaic Turner's syndrome [6], in cases with diminished ovarian volume following surgery, and has been used in siblings with discordant premature ovarian failure [7]. Women with BRCA1 mutation [8] and female-to-male transgender people [9] have also requested the preservation of their fertility. Although more controversial fertility preservation is being used to suspend the biological clock in women (social egg/tissue freezing) [10], this application will not be covered in this review.

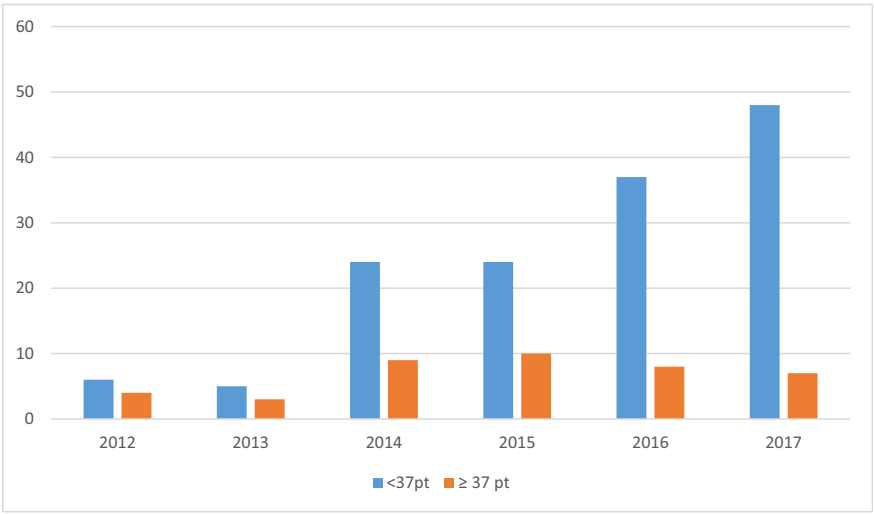
It is clear that there has been an increase in awareness of fertility preservation/oncofertility over time and that this is reflected in the number of patients cryopreserving material at well-established and centralised fertility preservation centres [1]. Our data also reflect this trend (Fig. 1) and identify the major types of cancers for which fertility preservation was performed (Fig. 2).

What material may be available?

In general, mature oocyte cryopreservation offers women the best option for achieving a future pregnancy using their own gametes, but this is not an option for children or adolescents. Previously, embryo cryopreservation was regarded as having a higher potential for pregnancy than oocyte cryopreservation but, as will be discussed in this review, this is no longer the case. Even when it was, the obstacle of requiring of a sperm donor and a quick decision often precluded embryo cryopreservation for single women. An important consideration also associated with embryo cryopreservation in the context of fertility preservation is the continuation of the relationship with the male partner, which is known to be more fragile following cancer than in the general population. This would suggest that the best option for storing female reproductive potential for future use is mature oocyte cryopreservation regardless of marital status.

For many women, the urgency to commence cytotoxic treatment does not permit adequate time for the cryopreservation of mature oocytes. In contrast, no delay is required for the cryopreservation of ovarian tissue, and this is the only option for prepubertal girls, although the subsequent potential for establishing pregnancy in this population is currently unknown. To maximise the potential for future pregnancy, if time permits, both ovarian tissue and oocytes can be cryopreserved. Oocytes collected during preparation of the ovarian tissue from small antral follicles are also cryopreserved at some clinics [11] although, again, their potential is unknown.

a



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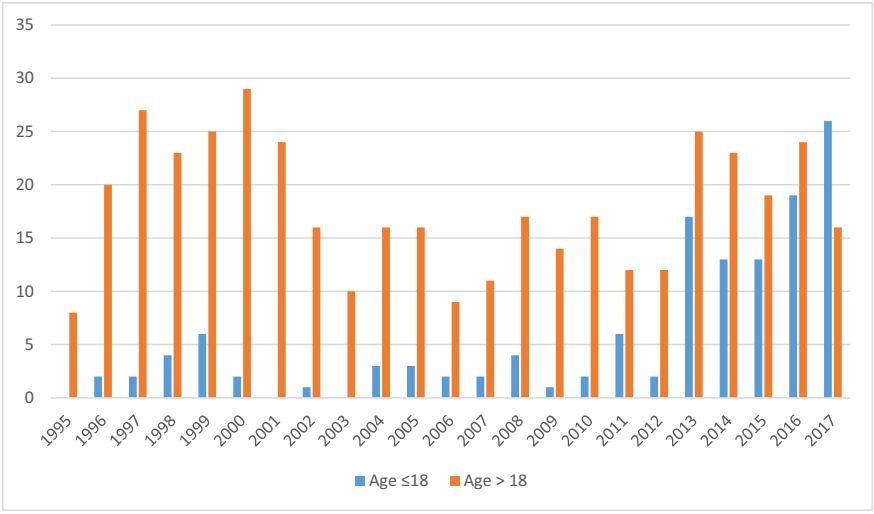


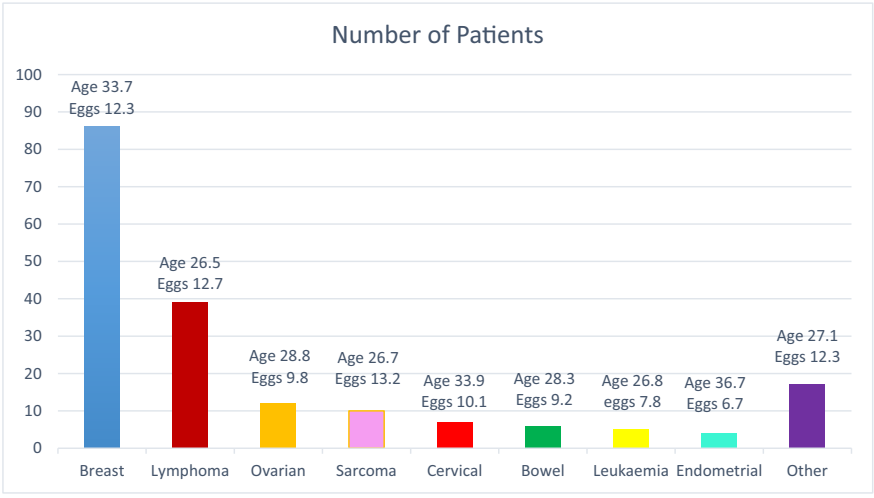
Fig. 1. a Increase in patients with cancer vitrifying oocytes with time at RWH/MIVF. b Number of patients cryopreserving ovarian tissue with time at RWH/MIVF.

Oocyte cryopreservation

Evolution of technology

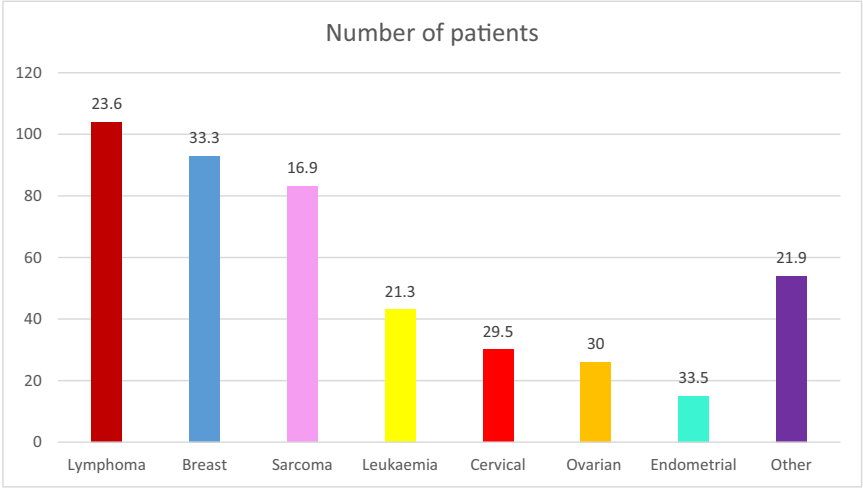
Subzero storage of animal gametes, both male and female, was first reported in the 1950s, but it was not until the 1980s that the first birth from a frozen human oocyte were reported [12]. At this time, animal studies raised major concerns regarding the risk of generating aneuploid embryos, because of the temperature sensitivity of the meiotic spindle involved in anchoring chromosomes, and the potential for the formation of abnormal foetuses. However, there were also concerns that the cryoprotectant used to freeze oocytes (dimethyl sulphoxide; DMSO) caused the artificial activation of nuclear

a



Mean age, mean number of eggs

b



Mean age

Fig. 2. a Type of cancers associated with oocyte vitrification. b Type of cancers associated with ovarian tissue cryopreservation.

material (parthenogenetic activation) and of cortical granules, thereby inhibiting fertilization ([13] reviewed). The above concerns brought clinical studies to an abrupt halt.

Clinical results for human embryos, using a different cryoprotectant (propanediol; PROH) in conjunction with a slow freezing rate, showed a great promise at that time and prompted our studies trialling this approach in mouse and human oocytes. Our subsequent results showed improved survival [14] relative to previous, low rates of abnormal activation [15], similar fertilization rates to fresh human oocytes, a normal complement of chromosomes following fertilization [16]and development to the blastocyst stage [17]. In 1994, the world's first egg bank was opened at the Royal Women's Hospital,

Melbourne, to provide a fertility preservation option for young women with malignant disease. The technology was then disseminated to Italy where legal restrictions on embryo cryopreservation were imminent, and the first birth was reported by Porcu et al. (1997) [18]. This was soon followed by a number of additional pregnancies and births ([13]*).

It became apparent during the early stages of clinical application that oocyte survival was variable, and improvement in the methodology for the dehydration of the oocyte before cryopreservation was necessary. Although increasing dehydration with higher sucrose (0.3 M) improved survival [19], implantation of the embryos that subsequently developed was approximately half that of embryos derived from fresh oocytes ([20]*). In contrast, milder dehydration approaches using 0.2 M sucrose together with modified rehydration protocols also showed improved survival, but, in these cases, equivalent implantation rates to those obtained with fresh oocytes could also be achieved [21].

The above developments were, however, concomitant with the development of an ultrarapid cryopreservation procedure (vitrification) involving the use of very high levels of cryoprotectant [22]. Significantly, modifications based on the demonstration that the toxicity associated with the high concentrations could be eliminated by using a mixture of cryoprotectants [23], saw the introduction of this technology first for embryos and subsequently for oocytes. Initially, spasmodic case reports of pregnancies from vitrified oocytes appeared in the literature [24], but these were viewed with some scepticism. However, two important clinical studies [25,26] soon started to change attitudes to vitrification.

Current effectiveness of technology

The initial results with vitrification were in the context of oocytes for an oocyte donation program [26] and therefore from young women (mean age 27 years). The high survival rates achieved with an open vitrification system in these oocytes (>90%) and the demonstration of equivalent clinical outcomes to those from fresh oocytes in relation to fertilization, embryo development and implantation in a large series of over 3000 oocytes [27] heralded the rapid acceptance of vitrification as the technique of choice for the cryopreservation of human oocytes. Although not all groups achieved equivalent high survival rates with donor oocytes or with oocytes from a wider age group, equivalent implantation rates to those obtained with fresh oocytes were consistently observed. Survival rates have been shown to be affected by the age of the women [28,29], and models have been developed to predict the number of oocytes required to achieve a live birth from vitrified oocytes in relation to age [28,29].

Evidence for safety of technology

Equivalent development and implantation rates with vitrified to those with fresh oocytes indicate that the high concentrations of cryoprotectants used and the procedures per se have no impact on subsequent events, except a slight delay in stage-specific development timings in the vitrified oocytes [30]. First trimester pregnancy loss with vitrified oocytes is reported to be similar to that with fresh oocytes [29], and it is estimated that thousands of babies have been born using vitrified oocytes. The obstetric and perinatal outcomes of over 800 pregnancies from vitrified oocytes compared to those from fresh oocytes show no difference in birth defects or major or minor malformations [31]. Similarly, a report showing no apparent increase in congenital anomalies in a study of over 900 babies from slow frozen oocytes [32] is encouraging although longitudinal follow-up is required.

The high vitrification survival rates were mostly achieved by extremely rapid cooling in direct contact with liquid nitrogen (open), thereby raising the issue of potential contamination during cooling and/or during storage [33]. Although this risk may be theoretical in ART with no reports of contamination, there are concerns that the risk may increase for oocytes in long-term storage for fertility preservation. Procedures have been developed to reduce the potential risk, and a number of closed vitrification tools have also been developed, but there has been a reluctance to use closed systems because of the potential impact of slower cooling rates on survival. Our own data using a closed system (rapid-i) have refuted the requirement for an ultrarapid cooling rate with human oocytes [34], thus establishing a safer storage system with similar success rates to fresh oocytes.

Although long-term storage in appropriate conditions does not impact on survival and outcome, there is a small risk of storage tank malfunction. This has occurred recently in two US clinics and reinforces the message that all tanks should have an alarm system fitted and that contingency plans to deal with tank rupture and eliminate the loss of frozen material should be in place.

Specific issues related to cancer

With changes in attitude to commencing ovarian stimulation in the luteal phase of the cycle, many fertility preservation patients who previously had insufficient time available before chemotherapy are now cryopreserving mature oocytes. The number of oocytes retrieved following stimulation negatively correlates with age and not the type of cancer when age adjusted, with the exception of ovarian cancers [35], and has been used in a patient as young as 13 years of age [36]. Although this approach is routinely used for young women requiring fertility preservation [35,37], there are few data on the embryo development and pregnancy outcomes for this group of women mainly because of the young age at time of fertility preservation and the delay in cure of the cancer. In a large centre offering fertility preservation for over 10 years, less than 5% have returned to use their oocytes [38]. In these, the survival appears to be lower and the implantation rate together with live birth rate per transfer appears to be reduced in younger women (<36 years) when compared to that in patients from a similar age group undergoing social oocyte cryopreservation. Clearly, larger studies are required to evaluate whether this persists, to establish whether it relates to previous chemotherapy and to assess perinatal outcomes.

Future developments – in vitro matured oocytes

While in vivo matured oocytes offer the ideal source of female gametes for fertility preservation, advances in knowledge of the interaction between the oocyte and associated follicular cumulus cells in the maintenance of meiotic arrest and the subsequent re-initiation of meiosis gained from animal studies [39] have also improved outcomes with in vitro maturation (IVM) of animal and human oocytes [40]. The concept has been applied to fertility preservation patients where urgent treatment is necessary [41] or where ovarian stimulation is contraindicated [42], and oocytes have been collected in both the follicular and luteal phases of the cycle [43]. Although there is some ambiguity concerning the precise definition of IVM cycles, a large study of cancer patients who underwent IVM cycles reported that AMH and antral follicle count are good predictors of the number of oocytes recovered in these patients and that half of the oocytes matured in culture. However, no subsequent developmental information was reported [43].

In fertility preservation patients, immature oocytes can also be aspirated from small antral follicles during the preparation of ovarian cortex and, following IVM, approximately 50% will mature [11]. Subsequent fertilization and embryo development have also been achieved and a live birth reported [44]. This approach has also resulted in live births in two patients with ovarian cancer [45,46], for whom no other options were possible. This also provides potential additional material in prepubertal patients, but although higher numbers of oocytes were recovered, a higher proportion degenerated and fewer matured in culture than those obtained from adults [47]. This technique has also been used in other cases where ovaries are removed such as female to male transgender [9] and BRCA1 mutation patients [8]. Although promising, research to improve maturation rates and ensure the normality of these oocytes is required.

Ovarian tissue cryopreservation

Advantages in certain cases

For prepubertal girls, young adolescent girls and those requiring urgent treatment, ovarian tissue storage is the only realistic fertility preservation option. It has, however, been suggested, based on the limited number of oocytes obtained from a single stimulation cycle, that cryopreserving ovarian tissue in young women may offer a higher prospect of future fertility than oocyte cryopreservation. Although an

invasive procedure is required to remove ovarian tissue, this can be performed in conjunction with other operative procedures that require an anaesthetic. It has also been suggested, because of the low numbers of primordial follicles and non-uniform distribution observed in the older ovary (such as that present in many breast cancer patients), that the cryopreservation of ovarian tissue may not provide a realistic option for fertility preservation in those cases and that an age limit of 35 years should be applied [48].

Development of technology

Conceptually, the cryopreservation of ovarian tissue is more complex than that of gametes or embryos, requiring preservation of multiple cell types, which vary in volume and water permeability. Essentially, ovarian tissue cryopreservation is more similar to organ cryopreservation than to that of gametes or embryos.

Early animal studies established the potential of ovarian tissue cryopreservation. A resurgence of interest in ovarian tissue cryopreservation stemmed from the birth of a lamb following cryopreservation and grafting of ovarian tissue reported by Gosden et al., 1994 ([49]*). Gosden's team and ourselves [50] had also begun investigations into the cryopreservation of human ovarian tissue. This in-depth assessment of morphological damage associated with different cryoprotectants and freezing rates [51] established the preservation potential of a propanediol-based method used in conjunction with a slow freezing rate. Subsequent development of antral follicles [52] and the presence of mature oocytes following the hCG trigger-induced maturation of human cryopreserved ovarian tissue grafted into an immunodeficient mouse system [53] validated the histological studies and demonstrated the preservation of subsequent developmental potential in primordial follicles. The highly reproducible development of antral follicles and the presence of mature oocytes, in stored ovarian tissue from multiple patients with various cancers using the same cryopreservation method, established the robust nature of the cryopreservation regimen. Furthermore, the studies demonstrated highly reproducible preservation of large numbers of primordial follicles within and between tissue harvested from multiple patients ([54]*). Similar evaluation had also been performed for other cryoprotectant regimens used in conjunction with a slow rate of cooling for human ovarian tissue; e.g. a DMSO procedure developed by Gosden and a method based on the cryoprotectant ethylene glycol. Clinical application, in the form of autologous transplantation of the stored tissue into patients whose cancer had been treated successfully, was the next step in the development of the approach.

Donnez et al. (2004) [55] reported the first birth following the transplantation of cryopreserved ovarian tissue. In this case, and in the majority of subsequent births ([56]*), the cryopreserved ovarian tissue was transplanted into the existing ovary (orthotopic). Because it is known that there is often a long delay in the resumption of cycling post chemotherapy [57,58], and even post bone marrow transplant preconditioning chemotherapy [59], the possibility that pregnancies may have arisen by the reactivation of residual native ovarian cortex, and not the cryopreserved tissue, cannot be excluded.

Evidence of follicle development and collection of oocytes following the transplantation of cryopreserved ovarian tissue at non-ovarian sites (heterotopic transplantation) clearly shows the function of the cryopreserved ovarian tissue ([60]*). Unequivocal evidence of successful preservation of full developmental potential in primordial follicles within cryopreserved ovarian cortex has been established with the birth of twins from tissue transplanted to an abdominal site in a patient who had undergone a bilateral oophorectomy [61]. This birth, and another from an oocyte collected from a heterotopic site (Gook unpublished), has resulted from tissue cryopreserved using the propanediol method. Similar evidence has also been reported for the ethylene glycol [62] and DMSO procedures [63]. Although success has been achieved with all methods, there is no comparative data to date and only little information on the relative efficiency of the methods presently being used for human ovarian tissue cryopreservation.

On the basis of evaluation in cynomolgus monkeys, vitrification has also been used for human ovarian tissue cryopreservation and two births have been reported from vitrified ovarian cortex [64].

Current evidence of effectiveness

Although proof of principle has been established, there remains a paucity of systematic information regarding the clinical efficiency of the cryopreservation of human ovarian tissue and

subsequent grafting. Notwithstanding this and the fact that the vast majority of ovarian tissue has been grafted either onto the ovary or close to an existing ovary, some general information can be gained from the world experience, which has now resulted in more than 130 births. Resumption of cycling generally occurs 3–4 months following grafting [56] regardless of the cryopreservation and grafting procedure, thus confirming the predicted follicle developmental timeline for recruitment and growth from the primordial follicle stage. In experienced clinics offering fertility preservation, those women in which resumption of cycling did not occur were over 37 years of age at the time the tissue was cryopreserved [65]. Failure to resume cycling following grafting at our centre has occurred only in tissue frozen at other centres and would appear to relate to poor cryopreservation (personal observation). Once initiated, regular cycling will continue until follicle numbers are exhausted. This is dependent on the follicular density and volume of tissue grafted and has continued for over 5 years in some cases (Table 1). The longevity of function in tissue grafted at the heterotopic site indicates that a large number of primordial follicles have survived the cryopreservation. The similarities in retrieval of oocytes, fertilization and embryo development from follicles developed at a heterotopic site to those from an orthotopic site (Gook unpublished) and also in the subsequent pregnancies (see above) further emphasise the preservation of developmental potential achieved with this cryopreservation regimen.

One population of patients that often requests the cryopreservation of ovarian tissue is prepubertal girls, and, again, there is a paucity of data on clinical effectiveness. Antral follicle development following the xenografting of cryopreserved prepubertal ovarian tissue has demonstrated preservation and developmental potential in this tissue [66]. Grafting initiated puberty in one patient [67], and live births were reported following the grafting of cryopreserved peri pubertal [68] and prepubertal tissue [69].

Without an international registry of the number of patients having cryopreserved ovarian tissue grafted, it is difficult to assess the clinical efficiency, and caution must also be exercised when assessing the proficiency of all clinics that are performing cryopreservation and grafting of ovarian tissue (see above) because wide variation in experience with relatively new technology could have significant impact on this data. An estimate based on five major centres (111 patients) indicated a pregnancy rate of 29% and live birth rate of 23% ([56]*). Data collected from eight centres (Table 2) suggested pregnancy and live birth rates of 47% and 34%, respectively.

Specific concerns relating to residual malignant cells within cryopreserved tissue

The relative risk of potential malignancy within the ovarian tissue has been reviewed for various cancers ([70]*) with high risk status identified in the cases of ovarian cancers and leukaemia (see later). However, the routine histological evaluation of ovarian tissue presenting for cryopreservation (irrespective of estimated risk) has detected malignancy in an area attached to the ovary that contained Hodgkin's lymphoma cells in one patient [71] and Ewing's sarcoma in the medullar area of the ovary in another patient ([72]*). Previous evaluation of multiple ovarian biopsies from patients with Hodgkin's lymphoma suggested this population to be at low risk of malignant contamination in the

Table 1
Longevity of ovarian function following the autografting of cryopreserved (slow cooled) ovarian tissue.

	Cryoprotectant	Patients	Graft Function (years)
Stern 2013 [61]	1.5 M PROH + 0.1 M Sucrose	1	5.4*#
		1	8#
Kim 2012 [93]	1.5 M DMSO + 0.1 M Sucrose	1	7#
Imbert 2014 [94]	1.5 M DMSO + 0.1 M Sucrose	1	7*
Liebenthron 2015 [95]	1.4 M DMSO	1	7*
Jensen 2015 [96]	1.5 M EG + 0.1 M Sucrose	2	≥10*
		9	≥5*
Silber 2015 [97]	1.5 M PROH + 0.2 M Sucrose	1	3.2

PROH propanediol, DMSO dimethyl sulphoxide, EG ethylene glycol, * achieved a pregnancy, # heterotopic graft.

Table 2
Number of patients receiving a graft and the resultant pregnancies from eight large centres.

	Cryoprotectant	Number of patients	Number with resumption of function	Number of pregnancies	Number of live births
Meirow 2016 [98]	1.5 M DMSO + 0.1 M Sucrose	20	20	14	10
Donnez 2015 [99]	1.5 M DMSO + 0.1 M Sucrose	13	10	6	6
Demeestere 2015 [68]	1.5 M DMSO + 0.1 M Sucrose	10	9	5	4
Diaz-Garcia 2018 [38]	1.5 M DMSO + 0.1 M Sucrose	44	43	15	10
Liebenthron 2015 [95]	1.4 M DMSO	68	43	16	12
Silber 2012 [97]	1.5 M PROH + 0.2 M Sucrose	11	11	8	6
Jensen 2015 [100]	1.5 M EG + 0.1 M Sucrose	41	41	24	14
Stern 2013 [61]	1.5 M PROH + 0.1 M Sucrose	26	20	4	5
		233	197	92	67

ovary [73]. Taken together, the above cases indicate that caution should be exercised when considering grafting of ovarian tissue in all patients regardless of expected risk. Furthermore, they emphasise the importance of testing of tissue before grafting and the need for patient awareness in relation to the unknown risk.

Xenografting of the ovarian tissue has been used for testing of tissue with an expected high risk of contaminating malignant cells, the rationale being that low numbers of malignant cells, if present, would proliferate with time and allow increased sensitivity with respect to detection. In a series of tissue samples from 10 patients with ovarian cancer, no evidence of malignancy was detected following xenografting [74]. To date, there have been four reported cases of autografting of ovarian tissue from patients with a malignant ovarian pathology [61,62,75]. All but one demonstrated resumption of ovarian function, conceived and had tissue removed immediately following delivery. In one case, reoccurrence was observed at time of caesarean section, although not at the graft site [76], and the possibility that this had arisen from the grafted tissue cannot be excluded.

Two studies examined the potential of leukemic contamination within ovarian tissue using the xenografting system [77,78]. In some cases, a leukemic marker could be detected in the tissue using PCR, indicating the presence of leukemic cells in the tissue. However, following xenografting, only some of the animals with this tissue developed malignancy. The lack of detection in known contaminated tissue is a likely consequence of the study design, attributable to the probable elimination of circulating malignant cells as a result of the immune leakage observed with the specific immunodeficient strain of mice used (this same strain was used in the ovarian cancer study), together with the fact that only one animal was used to test each patient's tissue. In contrast, all marker-positive tissue resulted in the detection of malignant cells when xenografted into a different mouse strain and when each patient's tissue was tested in multiple mice (Gook unpublished). These studies clearly suggest that tissue positive for malignant markers should not be returned to the patient. The previous studies referred to above also suggested that tissue harvested after chemotherapy did not harbour leukemic cells. One group recently decided that ovarian tissue harvested post-chemotherapy from a patient with leukaemia, and with no evidence of markers on PCR, was safe and consequently grafted tissue back into the patient. This resulted in two births, and there is no evidence of reoccurrence to date [79]. However, the high prevalence of detection of leukemic cells in mice xenografted with tissue taken after chemotherapy in our study suggests caution should, again, be exercised.

Future research

Further research is necessary to establish the risk of malignant cells within tissue from patients with leukaemia and whether these can be eliminated selectively from the tissue. Isolation of follicles free of

stromal cells may reduce contamination [80], and it has been postulated that, after the depletion of leukemic cells, the follicles could be reconstructed in a matrix to form a mini or artificial ovary and subsequently grafted to the patient. A number of matrix compositions are presently being evaluated (fibrin, fibrinogen, thrombin complex and decellularised ovary) [81,82]. It is important that appropriate model systems be used to test the potential effectiveness of these approaches. The challenges associated with the isolation of large populations of primordial follicles, free of leukemic cells [80], while retaining the potential for subsequent maturation into viable mature oocytes that are capable of initiating ovarian cyclicity, cannot be underestimated.

Taking the above possibility a stage further, an attractive alternative would be to grow follicles in the laboratory to a stage at which mature oocytes could be retrieved, thereby avoiding grafting of tissue. Follicles from a number of animal species have been grown in culture although these have mostly been follicles which, at the time of isolation, had already been recruited into the growing pool [83]. To maintain the three-dimensional structure, follicles are encapsulated in a permeable gel (alginate). This support structure has been a critical development for the long-term culture of follicles with some human follicles growing from 0.06 to 0.6 mm over 30 days in culture [84,85] and, in one case, production of a mature oocyte following the addition of the final maturation trigger [86]. Although not achieving the expected growth of in-vivo follicles, the ability of follicular mural granulosa, cumulus cells and oocytes to respond, to resume meiosis and to produce a mature oocyte is a remarkable breakthrough.

A live offspring derived from the in vitro culture of non-growing mouse follicles by Eppig and O'Brien (1996) [87] provided proof of principle for full IVM but not until recently has this been able to be repeated [88]. A two-step culture system starting with small fragments of human ovarian cortex permitted the development to the antral stage [89]. The combination of the above developments using the encapsulation of follicles in alginate and IVM has recently resulted in mature oocytes [90], again a significant development.

Much of the above progress has evolved from fundamental animal studies on the mechanism of inhibition of follicles that enter the growing pool, thus delaying their subsequent activation [91]. Addition of inhibitors to mimic the in vivo pathways during the culture of human ovarian tissue and subsequent grafting has resulted in a live birth in a patient with premature ovarian insufficiency [92].

Conclusion

Female fertility cryopreservation has evolved from the research phase into the present multifaceted approach, thereby offering options to women presenting with anticipated loss of fertility. Although many questions remain to be investigated, a review of the technical advances in this field that underpin clinical application gives an important insight into the field of female fertility preservation and highlights the challenges ahead.

Practical points

- Mature oocyte cryopreservation can now achieve results comparable to those from embryo cryopreservation, and, therefore, there is no longer a justification for creating embryos merely to ensure successful cryopreservation for fertility preservation patients.
- Births following the heterotopic grafting of cryopreserved ovarian tissue have established unequivocal clinical evidence of the successful cryopreservation of viable primordial follicles.
- At present, over 100 births have been reported following the grafting of cryopreserved ovarian tissue using slow freezing methods, and two births have also been reported from the grafting of vitrified ovarian tissue.

Research agenda

- In vitro maturation of oocytes collected during the preparation of ovarian tissue could provide additional stored reproductive potential for women, but the normality of the resultant oocytes and embryos needs to be established.
- Further validation of the cryopreservation of prepubertal ovarian tissue is necessary.
- More extensive evaluation of the potential risk of malignant contamination in ovarian tissue from patients with leukaemia and ovarian cancers, using appropriate xenografting models, is required to determine the safety of grafting such ovarian tissue.
- The in vitro development of isolated follicles, free of leukemic cell contamination and encased in an artificial ovary, shows promise and should be a focus for future research activity.

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Conflict of interest

The authors have no conflict of interest.

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