Female fertility preservation strategies: cryopreservation and ovarian tissue in vitro culture, current state of the art and future perspectives

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Summary

In the present review, the main strategies of female fertility preservation are covered. Procedures of fertility preservation are necessary for women who suffer from diseases whose treatment requires the use of aggressive therapies, such as chemotherapy and radiotherapy. These kinds of therapy negatively influence the health of gametes and their progenitors. The most commonly used method of female fertility preservation is ovarian tissue cryopreservation, followed by the retransplantation of thawed tissue. Another approach to female fertility preservation that has been actively developed lately is the ovarian tissue in vitro culture. The principal methods, advantages and drawbacks of these two strategies are discussed in this article.

Keywords: Cryopreservation, In vitro, Oncology, Ovary, Tissue.

Introduction

Female fertility preservation is one of the most common problems addressed by assisted reproductive technologies. Many women suffer from diseases like hormone-dependent malignancies (e.g. breast cancer) or autoimmune diseases (e.g. vasculitis), the treatment for which involves aggressive methods of therapy such as radiotherapy and chemotherapy. As these methods negatively influence the health of gametes and their progenitors, fertility preservation is required in these cases. Moreover, developing new techniques allowing us to preserve fertility may be necessary for women who want to postpone their childbearing.

Although oocyte cryopreservation is commonly used for fertility preservation, this method involves hormonal ovarian stimulation, which cannot be used in some groups of patients, e.g. prepubertal girls and patients who suffer from hormone-dependent types of cancer. This paper presents a review of the current literature and a discussion of the data regarding methods of fertility preservation that are applicable for patients who cannot undergo hormonal stimulation. These techniques refer to two main strategies of female fertility preservation that are being actively developed: ovarian tissue cryopreservation followed by retransplantation, and ovarian tissue in vitro culture.

Main strategies of female fertility preservation

Currently, patients who suffer from different kinds of hormone-dependent malignancies, such as breast cancer or autoimmune diseases like vasculitis, are offered an aggressive form of therapy, which negatively influences the health of both themselves and the gametes. For example, the treatment of breast cancer, which is a hormone-dependent kind of cancer, may involve a drastic method like oophorectomy. This
procedure leads to a decrease in the production of sex hormones (Di Leva Gianpiero et al., 2013). Moreover, treatment of other cancer types includes radio- and chemotherapy, which negatively affects the ovarian status. It is well known that most of the alkylating chemotherapeutic agents are gonadotoxic (Bokemeyer et al., 1994; Thomson et al., 2002). Use of these agents in a therapy protocol leads to the degradation of follicles and oocytes (Familiari et al., 1993; Oktem & Oktay, 2007a). Moreover, such treatment may result in the formation of ovarian tissue fibrosis (Meirow, 1999; Behringer et al., 2005). Chemotherapeutic agents induce apoptosis in ovarian stroma and in the follicular system (Verga Falzacappa et al., 2012), which leads to a reduction in follicle numbers or, in other words, results in an increase in the risk of infertility (Oktem & Oktay, 2007b).

The use of radiation therapy is dangerous for women over the age of 30, because the effective sterilizing dose of radiotherapy decreases with age (Wallace et al., 2005). At the same time, the risk of malignancy formation increases with age (Xu et al., 2013). Given these facts, the use of aggressive antitumour therapy for women over 30 must be supplemented with procedures of fertility preservation.

In contemporary society, many women prefer to delay childbearing, but the number of follicles decrease and reproductive health becomes impaired with age, reducing the probability of normal fertilization and pregnancy (Wallace & Kelsey, 2010). Therefore, methods that allow us to preserve fertility could also help these women to bear healthy children.

In some cases, fertility preservation is assisted with a classical cycle of ovulation induction (Cardozo et al., 2015; Sigismondi et al., 2015). Obtained oocytes can be cryopreserved, or fertilized and the developed embryos can subsequently be cryopreserved. Use of such a protocol is possible only in cases in which the woman is absolutely healthy or when her disease was diagnosed as being at an early stage of development and does not depend on gonadotropic and steroid sex hormones. Otherwise, the use of this protocol may lead to growth of the tumour. Moreover, such a strategy of therapy is unsuitable for prepubertal patients (Mahajan, 2015).

At the present time, a patient requiring fertility preservation is offered cryopreservation of ovarian tissue, followed by retransplantation of thawed fragments into the patient’s body (Meirow et al., 2007). However, technologies of ovarian tissue in vitro culture are being actively developed (Roy & Treacy, 1993; Abir et al., 1997, 1999, 2001; Nayudu et al., 2003; Xu et al., 2011b). The main aim of these technologies is to produce mature oocytes capable of fertilization from pieces of in vitro cultured ovarian tissue (Filatov et al., 2015). Embryos obtained after the fertilization of oocytes may be cryopreserved and transferred into the patient’s uterus after full recovery. Moreover, obtained embryos may be used in surrogacy programmes if the patient would like to have a child before the disease is cured.

**Diseases whose treatment requires fertility preservation**

Many diseases whose treatment requires aggressive methods of therapy and procedures of fertility preservation are known. These diseases can be divided into two groups: oncological and non-oncological diseases. Non-oncological diseases include autoimmune and haematological disorders that, like oncological ones, are treated with chemotherapy and radiotherapy (Donnez et al., 2006a,b, 2010a,b). The most common diseases the treatment of which requires procedures of fertility preservation are represented in Table 1.

In many cases, diagnosis of the disease occurs at a late stage, when treatment must begin immediately. In these situations, the usage of standard protocols of assisted reproductive technologies, followed by the cryopreservation of embryos obtained after in vitro fertilization of oocytes received via ovulation stimulation, is not possible (Sigismondi et al., 2015, Cardozo et al., 2015). Moreover, the use of classical programmes of assisted reproductive technologies for fertility preservation is not possible for prepubertal girls (Mahajan, 2015). In these cases, the application of ovarian tissue cryopreservation technologies or the ovarian tissue in vitro culture technique seems to be the best strategy for female fertility preservation.

**Methods of ovarian tissue isolation**

Isolation of ovarian tissue for further cryopreservation or in vitro culture may be performed in two ways: surgical removal of the whole ovary or biopsy of ovarian tissue pieces. The most commonly used method for obtaining ovarian tissue is laparoscopy (Meirow et al., 1999), but minilaparotomy also may be used for this purpose (Bolla et al., 2012). There is no consensus on the amount of ovarian tissue that must be left in the patient’s body after the procedure of ovarian tissue isolation. If treatment requires usage of an aggressive therapy that can negatively influence reproductive function, it is better to isolate larger parts of ovarian tissue (Rosendahl et al., 2008; Harel et al., 2011). Conversely, parts of ovarian tissue in the body must be preserved in order to provide the better survival of retransplanted fragments.

If the proposed fertility preservation strategy is the cryopreservation of ovarian tissue followed by retransplantation of isolated fragments, in most cases,
Table 1 Diseases that require fertility preservation (according to Wallace et al., 2005; Donnez & Dolmans, 2013)

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<thead>
<tr>
<th>Non-oncological</th>
<th>Oncological</th>
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<tr>
<td>Autoimmune:</td>
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<tr>
<td>Systemic lupus erythematosus</td>
<td>Osteosarcoma</td>
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<tr>
<td>Rheumatoid arthritis</td>
<td>Ewing sarcoma</td>
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<td>Behçet’s disease</td>
<td>Breast cancer</td>
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<td>Wegener’s granulomatosis</td>
<td>Cervical carcinoma</td>
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<td>Haematological:</td>
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<tr>
<td>Sickle-cell anaemia</td>
<td>Vaginal carcinoma</td>
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<tr>
<td>Thalassaemia major</td>
<td>Vulvar carcinoma</td>
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<tr>
<td>Aplastic anaemia</td>
<td>Ovarian tumours</td>
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<tr>
<td>Other:</td>
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<tr>
<td>Inflammatory process in ovaries and fallopian tubes</td>
<td>Hepatoblastoma</td>
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<tr>
<td>Amenorrhoea</td>
<td>Neuroblastoma</td>
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<tr>
<td>Multiple cysts’ formation in ovaries</td>
<td>Medulloblastoma</td>
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the cortical part of ovarian tissue is extracted. Cortical ovarian tissue contains the bulk of the follicles, particularly the primordial and primary ones. Furthermore, it is supposed that after the procedure of ovarian tissue cryopreservation and thawing, small follicles survive better than do larger ones (Hovatta et al., 1996, 1997; Newton et al., 1996; Oktay et al., 1997).

The ovarian tissue biopsy procedure allows the obtaining of fragments about 1 cm x 4–5 mm x 1–1.5 mm (Donnez et al., 2006a, 2010a, 2013). Before cryopreservation, isolated fragments must be separated into small pieces about 0.3–2 mm wide (Hovatta et al., 1996; Silber et al., 2005; Silber, 2011). There are two main reasons to cut obtained ovarian tissue fragments into thin slices. First, after retransplantation, thin tissue slices stimulate better neovascularization than thicker ones do. This situation allows a decrease in ischaemia stress in retransplanted tissue (Silber, 2011; Andersen et al., 2012; Silber & Barbey, 2012). Second, the thinness of fragments allows cryoprotectants to penetrate better into the tissue, resulting in less injury to the tissue during the procedures of freezing and thawing. It has been demonstrated that 2 mm thick slices thaw better than 4 mm thick slices (Ferreira et al., 2010).

In some cases, a whole ovary instead of ovarian tissue fragments can be used for retransplantation (Leporrier et al., 1987; Wang et al., 2002; Hilders et al., 2004; Martinez-Madrid et al., 2004; Mhatre et al., 2005; Imhof et al., 2006; Silber et al., 2008a,b). Whole ovary retransplantation decreases the risk of ischaemia in ovarian tissue because the immediate revascularization of the transplant is achieved via the vascular pedicle (Leporrier et al., 1987; Wang et al., 2002; Hilders et al., 2004; Martinez-Madrid et al., 2004; Mhatre et al., 2005; Imhof et al., 2006; Silber et al., 2008a,b). There is a clinical case of a live birth after the transplantation of a non-cryopreserved whole ovary into the donor’s body (Silber et al., 2008b). However, cryopreservation of the whole ovary seems to be impossible because, in the event of microvascular anastomosis failure, all ovarian tissue may be lost with no chance of future fertility restoration (Courbiere et al., 2009).

**Ovarian tissue cryopreservation techniques**

The two most commonly used methods of ovarian tissue cryopreservation are vitrification and slow freezing. The slow freezing technique was developed earlier than vitrification. The slow freezing protocol involves ovarian tissue impregnation with the cryoprotective agent, followed by a step-by-step temperature lowering. Usually, the slow freezing procedure involves the application of programmed freezers, which are special devices that allow the gradual lowering of temperature according to the program’s settings. In most cases, tissue is cooled to −140°C and then placed into liquid nitrogen for

Both slow freezing and vitrification require the exposure of ovarian tissue to a cryoprotective solution. The principal difference between vitrification and slow freezing is the very rapid cooling of the sample in the former. Rapid cooling allows the avoidance of ice crystal formation in the tissue. The appearance of ice crystals inside cells during cryopreservation has a detrimental effect on their viability: ice has a lower density than water, meaning that frozen water takes up more volume than the liquid form. Ice crystals can impair a cell’s membrane and, after thawing, a cell with a destroyed membrane may die.

The vitrification technique has demonstrated good results in the cryopreservation of oocytes and early embryos (Kuwayama et al., 2005; Cobo et al., 2009; Abedellhafez et al., 2010; Cobo et al., 2010; Edgar & Cook, 2012), but this method has grave disadvantages. Cryopreservation with a vitrification protocol requires the application of cryoprotective agents in high concentrations, which may negatively affect cryopreserved cells (Fahy et al., 1984; Isachenko et al., 2003, 2007; Gandolfi et al., 2006; Keros et al., 2009; Kagawa et al., 2009; Amorim et al., 2011). Moreover, the high concentration of cryoprotective agents can cause osmotic shock in cells (Fahy et al., 1990). Only two clinical cases of a successful pregnancy followed by a live birth after the vitrification and retransplantation of ovarian tissue are known (Kawamura et al., 2013; Suzuki et al., 2015). Brief descriptions of cryopreservation protocols whose usage led to pregnancies and live births are listed in Table 2. Comparison of the effectiveness of vitrification vs. slow freezing requires further studies, which are to be carried out in the near future.

Methods of ovarian tissue transplantation

There are three principally different methods of ovarian tissue transplantation: orthotopic retransplantation, heterotopic retransplantation and xenotransplantation. During orthotopic retransplantation, ovarian tissue is placed into the remaining ovary or (if that is not possible) into the pelvic cavity. In other words, ovarian tissue is transplanted into its native surroundings. In the case of heterotopic retransplantation, ovarian tissue is transplanted to a different site, e.g., under the skin of the forearm or under the abdominal wall (Oktay & Karlikaya, 2000; Callejo et al., 2001; Oktay et al., 2001; Radford et al., 2001; Donnez et al., 2004, 2005; Meirow et al., 2005; Schmidt et al., 2005; Demeestere et al., 2006; Kim et al., 2009; Grynberg et al., 2012).

At present, two main orthotopic retransplantation techniques of ovarian tissue exist. In a case where some parts of the ovary remain in the patient’s body, thawed pieces of cortical ovarian tissue can be attached to the remaining medulla. Where the medulla has not been saved, ovarian tissue pieces can be placed into the peritoneal window, in the region of small retroperitoneal vessels (Donnez et al., 2012, 2013). The main advantage of orthotopic retransplantation as compared with the heterotopic method is that natural conception may occur when ovarian tissue is transplanted orthotopically. Moreover, in the case of the orthotopic technique, fragments are placed into an environment that is native for ovarian tissue. It is likely that tissue retransplantation into its original physiological surroundings may promote the development of transplanted tissue more effectively than retransplantation into a heterotopic site. The main disadvantage of orthotopic retransplantation is that it is often impossible to determine whether ovulation has occurred in the transplanted tissue or in the remaining ovary fragment, especially in the event of a natural conception.

Heterotopic retransplantation of ovarian tissue has other advantages over the orthotopic method. Firstly, it is easy to observe the actual condition of transplanted tissue. Secondly, heterotopic retransplantation of ovarian tissue does not require the usage of general anaesthesia. Thirdly, it is easier (when required) to remove the transplanted fragment than it is in the case of orthotopic retransplantation.

However, heterotopic retransplantation also has some principal drawbacks. First, conception may occur only with assisted reproduction technologies; moreover, the non-native surroundings of transplanted tissue probably reduce the ability to induce neovascularization in the ovarian fragment than in the case of orthotopic retransplantation. Taken together, these two factors reduce the probability of pregnancy (Oktay et al., 2001, 2004; Partridge et al., 2004; Sonmezer & Oktay, 2004; Kim et al., 2009; Jemal et al., 2010; Donnez et al., 2013).

The method of Rosendahl et al., (2006) shows that ovarian tissue xenotransplantation can also be applied. In this case, ovarian tissue is transplanted under the skin or under the renal capsule of immunodeficient mice (Sonmezer & Oktay, 2004; Chao et al., 2008; Lan et al., 2010). Immunodeficient mice do not develop an immune response to tissues transplanted from other species (Bosma et al., 1983).

The main advantage of xenotransplantation is that the transplanted tissue is located outside the patient’s body. In the case in which malignant cells have remained in the transplanted tissue, the tumour will grow in the mouse rather than in the patient. For this reason, this technique seems to be very
Table 2 | Protocols of cryopreservation procedures that led to live births after the retransplantation of thawed ovarian tissue

<table>
<thead>
<tr>
<th>No.</th>
<th>Vitrification/Slow freezing</th>
<th>Cryoprotectors</th>
<th>Thawing</th>
<th>Reference</th>
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<tr>
<td>1</td>
<td>Slow freezing: cooling 2°C/min to −9°C, 5 min of soaking in cryoprotective solution, then manual seeding for ice crystal nucleation induction, 0.3°C/min to −40°C, 10°C/min to −140°C. Then samples were plunged into liquid nitrogen at −196°C. A programmable Planner freezer (Planner K10, Planner Ltd, UK) was used</td>
<td>Before the slow freezing procedure, pieces of tissue were transferred to 30 ml of 0.1 mol/l sucrose and 1.5 mol/l ethylene glycol in a PBS solution, and equilibrated for 30 min at 1°C on a tilting table</td>
<td>Samples were thawed rapidly in a 37°C water bath</td>
<td>Schmidt et al., 2003; Andersen et al., 2008</td>
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<td>2</td>
<td>Slow freezing: 2°C/min to −8°C; at this temperature, ice crystal nucleation was induced manually, followed by 0.3°C/min to −30°C, 50°C/min to −150°C, before being stored in liquid nitrogen. A programmable Planner freezer (Planner K10, Planner Ltd, UK) was used</td>
<td>Tissue slices were rinsed briefly in a PBS solution containing 10 mg HSA per ml (base medium) followed by dehydration in 1.5 mol/l propanediol with 0.1 mol/l sucrose in a base medium at room temperature for 90 min</td>
<td>Samples were transferred to a water bath (+37°C) and thawed for 2–3 min</td>
<td>Gook et al., 1999, 2005; Stern et al., 2013</td>
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<td>3</td>
<td>Slow freezing: cooling 2°C/min from 0°C to −8°C; ice crystal nucleation was induced manually by using forceps pre-cooled in liquid nitrogen; cooling 0.3°C/min to −40°C; cooling 30°C/min to −150°C. Then samples were stored in liquid nitrogen. A programmable Planner freezer (Planner K10, Planner Ltd, UK) was used</td>
<td>Fragments of tissue were placed into a Leibovitz medium containing 4 mg/mL human serum albumin and 1.5 mmol/L DMSO</td>
<td>Sample thawing was performed in the following way: at room temperature for 2 min, followed by ~2 min in a water bath (+37°C). Ovarian tissue pieces were rinsed in a Leibovitz medium three times</td>
<td>Donnez et al., 2004</td>
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<td>4</td>
<td>Slow freezing: the temperature was lowered from 4°C to −9°C at the rate of 2°C/min. After a manual seeding by touching the CryoTubes with liquid nitrogen pre-chilled forceps, samples were cooled to −40°C at the rate of 0.3°C/min, then to −140°C at 10°C/min. After stabilization of the temperature for 10 min, the samples were transferred to liquid nitrogen and stored until thawing. A programmable freezer (Planner Kryo 360/3.3) was used</td>
<td>Ovarian tissue pieces were cryopreserved in a solution of Leibovitz L-15 medium containing 1.5 mol/L dimethyl sulfoxide (DMSO), 0.1 mol/L sucrose and 10% decomplemented patient serum</td>
<td>After rapid thawing, strips were washed in decreased solutions of DMSO 1.5M (5 min), 1M (5 min), 0.5M (10 min) and in a solution of 0.05 mol/L sucrose in a Leibovitz L-15 medium supplemented with 10% decomplemented patient serum. The strips were then rinsed and transferred to the operating theatre for the graft in medium containing only 20% serum</td>
<td>Fauque et al., 2007; Roux et al., 2010</td>
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<td>No.</td>
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<td>5</td>
<td>Slow freezing: −2°C/min to 1°C, −0.5°C/min to −5°C, −0.3°C/min to −9.3°C, 10 min of soaking in a cryoprotective solution. Then −0.3°C/min to −40°C and −10°C/min to −140°C, at which temperature the samples were plunged into liquid nitrogen at −196°C. The open freezing system CTE-920 was used.</td>
<td>Pieces of ovarian tissue were equilibrated for 30 min in 1.5 mol/L ethylene glycol (or DMSO, or propanediol) and 0.1 mol/L sucrose in PBS solution on a tilting table on ice.</td>
<td>Thawing occurred rapidly in a warm water bath (+37°C). The tissue fragments were released from the protective cryopreservation medium in reverse order, with the addition of 0.25 mol/L sucrose.</td>
<td>Dittrich et al., 2015</td>
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<td>6</td>
<td>Slow freezing: after 5 min for equilibration at +4°C, cooling was performed at 0.5°C/min to −7°C, at which temperature seeding was automatically induced. Ovarian tissue was further cooled at a rate of 0.5°C/min to −50°C and subsequently at 5°C/min to −80°C, and finally at 8°C/min to −120°C. The tissue was then transferred to liquid nitrogen (−196°C) and stored until autografting. The programmable freezer CM25 was used.</td>
<td>The tissue was equilibrated at 4°C for 10 min in a 0.7M dimethyl sulphoxide solution containing 20% human serum in an RPMI 1640 medium. Samples were then transferred to the freezing solution with a similar composition, but one containing 1.5M dimethyl sulphoxide, and were left to equilibrate for 10 min at +4°C.</td>
<td>Thawing was performed in a water bath at +37°C until the ice was melted. The freezing medium was stepwise diluted over 7 min at +4°C to a final concentration of 0.14 M dimethyl sulphoxide, by adding a double volume of RPMI 1640 medium supplemented with 4% of HSA each minute. Ovarian tissue was then equilibrated over 3 min at +4°C, before replacing the diluted medium with the Flushing Medium. Thereafter, the ovarian tissue and Flushing Medium were equilibrated for 25 min at +37°C in a 5% solution of CO₂ to allow a more complete cell rehydration before grafting.</td>
<td>Callejo et al., 2001, 2013</td>
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<td>7</td>
<td>Slow freezing: samples were cooled from +1°C at a rate of −2°C/min to −9°C. After manual seeding, cooling at a rate of −0.3°C/min was performed down to −40°C, followed by rapid cooling at a rate of −10°C/min down to −140°C. Samples were then transferred to liquid nitrogen. The programmable freezer Kryo10 was used.</td>
<td>Ovarian tissue pieces were placed into the pre-cooled (+4°C) cryoprotectant solution: Leibovitz L-15 medium containing 1.5 M dimethyl sulphoxide (DMSO), 0.1 M sucrose and 10% (vol/vol) HSA.</td>
<td>The vials with samples were thawed individually by agitating them in water at room temperature (~100°C/min). The cryoprotectant was quickly removed from the tissue by repeated washing in saline and a fresh quantity of the Leibovitz medium.</td>
<td>Newton et al., 1996; Revel et al., 2009, 2011</td>
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Table 2 Continued

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<th>No.</th>
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<th>Cryoprotectors</th>
<th>Thawing</th>
<th>Reference</th>
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<td>8</td>
<td>Vitrification: the straw with sample was immersed vertically into liquid nitrogen (−196°C) for cooling</td>
<td>The vitrification solution (VS) contained H199 supplemented with 20% SSS, 5.64 M ethylene glycol, 5% (w/v) polyvinylpyrrolidone and 0.5 M sucrose. Three steps for the equilibration of ovarian tissue were used. Samples were placed in H199 supplemented with 20% SSS and 1.61 M ethylene glycol for 10 min, transferred to H199 supplemented with 20% SSS and 3.22 M ethylene glycol for 10 min and equilibrated in VS for 5 min. All procedures were performed at room temperature (±24–26°C). After equilibration in 1 ml VS for 5 min at room temperature, 0.2 ml of ovarian tissue cubes and vitrification solution were packed into 0.5 ml straws. Both ends of the straw were sealed</td>
<td>For warming, straws were warmed in water at +35°C for 10 s and the tissue cubes were expelled into 1 ml of H199 containing 20% SSS and 0.8 M sucrose (pre-warmed at +37°C). The tissue cubes were transferred to H199 containing 20% SSS and 0.4 M sucrose, and then to H199 containing 20% SSS for twice 5 min each at room temperature. After removal of the cryoprotectant, the ovarian sections were kept in H199 containing 20% SSS until transplantation</td>
<td>Suzuki et al., 2012; Kawamura et al., 2013</td>
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DMSO, dimethyl sulphoxide; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N′,N′,N′-tetra-acetic acid; HSA, human serum albumin; PBS, phosphate-buffered saline; SSS, serum substitute supplement.

promising for patients who suffer from hormone-dependent types of cancer. Furthermore, it is very simple to realize monitoring of transplanted tissue and to obtain mature oocytes. However, the application of xenotransplantation in routine clinical practice is not possible, due to the insufficiency of data about disease transmission from animals to humans via ovarian tissue (Seli & Tangir, 2005). Thus, the ovarian tissue xenotransplantation technique may be applicable only in research studies until the question of infection transmission is resolved.

Orthotopic, heterotopic and xenotransplantation methods have several advantages and drawbacks, as discussed above. In summary, orthotopic ovarian tissue retransplantation currently seems to be more optimal than the other techniques. In the case of orthotopic retransplantation, a tissue fragment is placed in a native environment with adequate temperature, blood pressure, mechanical tension and normal hormone and paracrine regulation. Furthermore, in contrast with heterotopic retransplantation and xenotransplantation, natural conception may occur in the case of orthotopic retransplantation. At the present time, xenotransplantation cannot be used in routine clinical practice, and the effectiveness of heterotopic retransplantation still remains unclear: there is only one case of a live birth after IVF procedures with an oocyte obtained from heterotransplanted ovarian tissue (Stern et al., 2013). In all other known cases of live births after ovarian tissue retransplantation, the orthotopic technique was used.

**Effectiveness of ovarian tissue cryopreservation**

The effectiveness of cryopreservation and thawing procedures can be estimated using different methods. Evaluation of a tissue’s condition after cryopreservation and thawing can be performed via histological analysis. However, the only reliable way to prove that
a cryopreservation and thawing technique is effective is to obtain mature oocytes capable of fertilization and further development, followed by the live birth of healthy baby.

At present, more than 25 cases of successful ovarian tissue cryopreservation that led to childbirth are known (Donnez et al., 2004, 2013; Meirw et al., 2005; Chung et al., 2013). Most of these cases are listed in Table 3. However, although the data are very promising, it is not known exactly whether the oocytes ovulated from the transplanted ovarian tissue or the remaining fragment of the ovary. In most cases, pieces of ovary remain in the patient’s body after an ovarian tissue biopsy, which is required for better survival rates of retransplanted tissue. It is only in isolated instances that women need a total bilateral oophorectomy, so only in these cases is it possible to declare confidently that an embryo has developed successfully from an oocyte obtained from the thawed ovarian tissue.

Cryopreservation of ovarian tissue followed by retransplantation is not applicable in several cases. Many scientific groups (Meirw et al., 2005; Silber et al., 2005; Donnez et al., 2006a, 2010a, 2013; Andersen et al., 2008; Sanchez et al., 2008; Schmidt et al., 2011; Silber, 2011) agreed that, as a rule, 35 is the upper age limit for the cryopreservation of ovarian tissue. This situation is due to the fact that, after the procedure of cryopreservation of ovarian tissue, follicles are preserved mainly in the early stages of folliculogenesis (primary and primordial follicles), the number of which reduces significantly with age.

In the case of prepubertal girls, obtaining ovarian tissue, followed by its cryopreservation and retransplantation, will negatively influence the patient’s health. Such procedures will provoke harmful hormonal stress. In these cases, it seems better to obtain mature oocytes from ovarian tissue cultured in vitro and use them in surrogate programmes.

In some instances, ovarian tissue cryopreservation cannot be used (for example, when the patient is a prepubertal girl) or is not very effective (when the patient is older than 35). In these cases, the ovarian tissue in vitro culture technique can be applied for female fertility preservation.

**Ovarian tissue in vitro culture**

There are two methods for an ovarian tissue in vitro culture technique: (i) two-dimensional (2D); and (ii) three-dimensional (3D) systems (Hirao, 2012). The principal drawback of 2D systems is the low correspondence between the microenvironment of the cultured fragment and the in vivo conditions. In the case of the 2D method, the ovarian tissue culture’s stroma and follicular cells migrate along the plane, follicles lose their normal shape and oocytes lose their normal cellular environment. Regulation of the 2D system’s conditions may be performed only by adding various chemical agents (growth factors, hormones, etc.) to the culture medium. Three-dimensional systems allow us to regulate the condition of cultivated ovarian tissue, not only by adding different chemical agents but also by variation of microenvironmental physical properties.

For in vitro culture, the whole ovary (Jin et al., 2010), ovarian fragments or single follicles can be used (Xu et al., 2006b). In vitro culture of a whole ovary or ovarian fragments has a significant disadvantage. This is due to the difficulties of nutrient delivery and oxygenation in the deep layers of cultivated tissue. Therefore, studies dedicated to isolated ovarian follicles in an in vitro culture seem to be the most effective. In the case of isolated ovarian follicles in vitro culture, all cultivated follicles receive required nutrients that penetrate into all of the follicular compartments via diffusion. Moreover, when single ovarian follicles are cultivated, it is possible to trace the development of each follicle, which is not possible in the cases of a whole ovary or ovarian tissue fragments in vitro culture.

Mammalian ovarian follicles in vitro culture can be performed using various substrates, e.g. agarose, collagen, Matrigel or alginites and their derivatives. The main parameters of different substrates used for ovarian follicles in vitro culture are listed in Table 4. Described substrates belong to the group of hydrogels, which are polymeric compounds that are powdery when dried. If water or another solvent is added to the dry hydrogel, its granules swell, resulting in the formation of a gel-like substance. This gel can be used further for the in vitro culture of various cell types, particularly for the culture of ovarian follicles.

The described substrates have various limitations in their applications. For instance, agarose gelaion requires a high temperature but heating is very harmful to ovarian follicles. The most commonly methods used for hydrogel gelaion (exposure to UV radiation, high temperatures and aggressive chemical treatment) are dangerous for the living objects.

Alginate hydrogel gelaion does not require toxic agents or application of non-physiological conditions (high temperature, UV radiation, etc.). Only the supplementation of a solution containing cross-linking agents (Ca²⁺ or Mg²⁺ ions) is needed for alginate polymerization. The effect of the conditions of hydrogel gelaion on living cells is less negative. This is because alginate hydrogel gelaion does not require temperatures higher than 37°C (physiological temperature) and polymerization agents (divalent cations, added to most culture mediums) have no
Table 3 Cases of successful ovarian tissue cryopreservation that led to live birth

<table>
<thead>
<tr>
<th>No. of patient</th>
<th>Age at cryopreservation/age at ovarian tissue transplantation</th>
<th>Disease</th>
<th>Chemotherapy before ovarian tissue cryopreservation</th>
<th>Transplantation type</th>
<th>Conception type</th>
<th>Note</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25/32</td>
<td>Granulosa cell tumour</td>
<td>No</td>
<td>Heteropic</td>
<td>IVF</td>
<td>Bilateral oophorectomy. Fertilization by ICSI. Dichorionic diamniotic twins</td>
<td>Stern et al., 2013</td>
</tr>
<tr>
<td>2</td>
<td>25/29</td>
<td>Hodgkin’s lymphoma</td>
<td>No</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Donnez et al., 2004</td>
</tr>
<tr>
<td>3</td>
<td>28/30</td>
<td>Non-Hodgkin’s lymphoma</td>
<td>Yes</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Singleton</td>
<td>Meirow et al., 2005</td>
</tr>
<tr>
<td>4</td>
<td>24/28 and 30</td>
<td>Hodgkin’s lymphoma</td>
<td>Yes</td>
<td>Orthotopic and heterotopic</td>
<td>Spontaneous</td>
<td>Unilateral oophorectomy was performed. Two singletons childbirths have been observed after orthotopic ovarian tissue transplantation</td>
<td>Demeestere et al., 2007, 2010</td>
</tr>
<tr>
<td>5</td>
<td>26/28</td>
<td>Hodgkin’s lymphoma</td>
<td>Yes</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Unilateral oophorectomy. Singleton</td>
<td>Andersen et al., 2008</td>
</tr>
<tr>
<td>6</td>
<td>27/30</td>
<td>Ewing sarcoma</td>
<td>No</td>
<td>Orthotopic</td>
<td>IVF and spontaneous</td>
<td>Whole left ovary and two-thirds of the right ovary were removed. Two singletons</td>
<td>Andersen et al., 2008; Ernst et al., 2010</td>
</tr>
<tr>
<td>7</td>
<td>20/22</td>
<td>Sickle-cell anaemia</td>
<td>No</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Roux et al., 2010</td>
</tr>
<tr>
<td>8</td>
<td>36/38</td>
<td>Breast cancer</td>
<td>No</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Fertilization by ICSI. Twins</td>
<td>Sánchez-Serrano et al., 2010</td>
</tr>
<tr>
<td>9</td>
<td>17/25</td>
<td>Metastatic neuroectodermic tumour</td>
<td>No</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Donnez et al., 2011</td>
</tr>
<tr>
<td>10</td>
<td>20/23</td>
<td>Hodgkin’s lymphoma</td>
<td>No</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Donnez et al., 2011</td>
</tr>
<tr>
<td>11</td>
<td>27/31</td>
<td>Microscopic polyangiitis</td>
<td>Yes</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Singleton</td>
<td>Donnez et al., 2011</td>
</tr>
<tr>
<td>12</td>
<td>27/32</td>
<td>Hodgkin’s lymphoma</td>
<td>Yes</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Dittrich et al., 2015</td>
</tr>
<tr>
<td>13</td>
<td>28/31</td>
<td>Breast cancer</td>
<td>No</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Dittrich et al., 2015</td>
</tr>
<tr>
<td>14</td>
<td>35/37</td>
<td>Hodgkin’s lymphoma</td>
<td>No</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Dittrich et al., 2015</td>
</tr>
<tr>
<td>15</td>
<td>30/35</td>
<td>Ovarian cancer</td>
<td>No</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Dittrich et al., 2015</td>
</tr>
<tr>
<td>No. of patient</td>
<td>Age at cryopreservation/age at ovarian tissue transplantation</td>
<td>Disease</td>
<td>Chemotherapy before ovarian tissue cryopreservation</td>
<td>Transplantation type</td>
<td>Conception type</td>
<td>Note</td>
<td>Reference</td>
</tr>
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<td>---------------</td>
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<td>---------------------------------------------------</td>
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<td>-----------</td>
</tr>
<tr>
<td>16</td>
<td>21/29</td>
<td>β-Thalassaemia</td>
<td>Yes</td>
<td>Orthotopic</td>
<td>Spontaneous</td>
<td>Singleton</td>
<td>Revelli et al., 2013</td>
</tr>
<tr>
<td>17</td>
<td>20/30</td>
<td>Dermoid cysts</td>
<td>No</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Bilateral oophorectomy. Fertilization by ICSI. Singleton</td>
<td>Callejo et al., 2013</td>
</tr>
<tr>
<td>18</td>
<td>19/23</td>
<td>Transfusion-dependent but well chelated thalassaemia</td>
<td>No</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Singleton</td>
<td>Revel et al., 2011</td>
</tr>
<tr>
<td>19</td>
<td>23/31, 32, 36</td>
<td>Ewing sarcoma</td>
<td>No</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Singleton</td>
<td>Rodriguez-Wallberg et al., 2015</td>
</tr>
<tr>
<td>20</td>
<td>18/26</td>
<td>Tubo-ovarian abscesses</td>
<td>No</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Both ovaries and tubes were removed. Fertilization by ICSI Singleton</td>
<td>Donnez et al., 2012</td>
</tr>
<tr>
<td>21</td>
<td>29/29</td>
<td>Amenorrhoea</td>
<td>No</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Both ovaries were removed. Fertilization by ICSI Singleton</td>
<td>Kawamura et al., 2013</td>
</tr>
<tr>
<td>22</td>
<td>24/26</td>
<td>Thyroid cancer</td>
<td>No</td>
<td>Orthotopic</td>
<td>IVF</td>
<td>Singleton</td>
<td>Kiseleva et al., 2014</td>
</tr>
</tbody>
</table>

ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.
Table 4 Substrates used for in vitro ovarian follicles’ culture

<table>
<thead>
<tr>
<th>Material</th>
<th>Origin</th>
<th>Method of gelation</th>
<th>Method of digestion</th>
<th>Interactions with cells</th>
<th>Organism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Animal</td>
<td>Heating</td>
<td>Collagenase</td>
<td>Binds to integrins</td>
<td>Mouse</td>
<td>Itami et al., 2011; Mochida et al., 2013; Sharma et al., 2009.</td>
</tr>
<tr>
<td>Matrigel</td>
<td>Animal</td>
<td>Heating</td>
<td>Collagenase</td>
<td>Binds to integrins</td>
<td>Cow</td>
<td>Oktem &amp; Oktay, 2007c; Oktem et al., 2011; Guzel et al., 2014</td>
</tr>
<tr>
<td>Agarose</td>
<td>Plant</td>
<td>Heating</td>
<td>Ionic cross-linking</td>
<td>None</td>
<td>Dog/Cat</td>
<td>Guzel et al., 2014; Fujihara et al., 2013.</td>
</tr>
<tr>
<td>Alginate</td>
<td>Plant</td>
<td>Ionic cross-linking</td>
<td>Alginate lyase or EGTA</td>
<td>None</td>
<td>Mouse</td>
<td>Xu et al., 2006a; Choi et al., 2014; Skory et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in the presence of divalent cations (i.e. Ca^{2+})</td>
<td></td>
<td></td>
<td></td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rhesus monkey</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Baboon</td>
</tr>
</tbody>
</table>

EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid.

toxic effects on cells. Moreover, cultivated cells do not form contacts with an alginate hydrogel. The alginate hydrogel destruction procedure does not impair cultivated follicles, because substances that digest the alginate hydrogel are unable to destroy cell contacts.

One of the principal advantages of alginate substrates is their vegetative origins. Therefore, alginate hydrogels can be used in ‘animal-free’ projects. In some studies, e.g. human ovarian follicle culture, the application of substances of animal origin is forbidden because it may lead to the contamination of the culture medium by viruses, proteins and other molecules that are able to interact with the cultivated tissue. There is no consensus on horizontal gene transfer when tissues or substances from different species are cocultured.

**Effectiveness of ovarian tissue in vitro culture**

To date, the ovarian tissue in vitro culture technique is not a widely used technique in clinical practice because the developing methods are far from perfect. Furthermore, there are no standardized technique protocols for effectively obtaining oocytes capable of in vitro fertilization. There are two principally distinct strategies of ovarian tissue in vitro culture: in vitro culture of ovarian cortical tissue fragments (strips) and in vitro culture of isolated ovarian follicles.

The cortical compartment of an ovary contains a large number of primordial follicles. Most commonly, ovarian tissue fragments are obtained by dissection of the cortical tissue into strips, with their subsequent culturing in vitro. This approach allows to support the growth of primordial follicles (Telfer et al., 2008).

Another approach to culture of ovarian tissue in vitro is based on the culture of isolated ovarian follicles. Commonly, primary and secondary follicles are used for this technique. For the ovarian tissue in vitro culture technique, researchers use various substrates that allow the stimulation of follicle growth and oocyte maturation, e.g., alginate (Xu et al., 2006a, 2009, 2011b; Amorim et al., 2009; Hornick et al., 2012; Choi et al., 2014; Skory et al., 2015), collagen (Sharma et al., 2009; Itami et al., 2011; Mochida et al., 2013), Matrigel (Oktem & Oktay, 2007c; Oktem et al., 2011; Guzel et al., 2014) and agarose (Fujihara et al., 2012). There are promising results showing that the ovarian tissue in vitro culture technique allows not only to stimulate follicular growth but to obtain mature oocytes that are competent for development after fertilization (Xu et al., 2006b, 2011a, 2013; Shikanov et al., 2009, 2011; Jin et al., 2010; Choi et al., 2014).
mature oocytes that are able to undergo successful fertilization and further development. Furthermore, after the transfer of these embryos to a surrogate mother, the live birth of healthy pups has been obtained (Xu et al., 2006b). However, live births of healthy pups after in vitro culturing of ovarian tissue have been shown in only a few studies (Yamamoto et al., 1999; Hasegawa et al., 2006; Xu et al., 2006b; Mochida et al., 2013; Higuchi et al., 2015). Such significant results motivate researchers to develop more advanced systems that will allow us to obtain mature oocytes more effectively.

The latest studies of the ovarian tissue in vitro culture technique are dedicated to the reconstruction of the ovarian corticomedullary structure. During folliculogenesis, in vivo growing follicles migrate from the more rigid ovarian cortex to the soft ovarian medulla (Xu et al., 2013). Therefore, in a natural environment, mechanical stress reduction occurs in follicular cells. Reconstruction of the corticomedullary ovarian structure in vitro can be performed using combined hydrogels; for example, a fibrin-alginate hydrogel can be used (Shikanov et al., 2009, 2011; Jin et al., 2010). A growing follicle secretes proteases that destroy fibrin, resulting in mechanical stress decreasing around the follicle and thus promoting its development (Shikanov et al., 2011).

Another approach to reconstruct the ovarian corticomedullary structure in vitro is using an alginate-collagen system. The drop in which a single follicle is cultivated contains two compartments. The centre of the drop consists of collagen, and its periphery is formed with alginate. This technique allows to create a system with heterogenic mechanical tensions. Collagen is softer than alginate, so collagen imitates the medulla and alginate imitates the cortex (Choi et al., 2014). It has been shown that follicular development up to the late antral stage occurs in 28% of cases when an alginate–collagen system is used (Choi et al., 2014). Preparation of such a complex culture system can be performed using microfluidic technologies (Streets & Huang, 2014). These technologies allow to create drops consisting of various substances in the required proportions by using directed microflows of liquids. The type of microflow is determined by the microfluidic chip configuration.

Technologies of 3D ovary reconstruction in vitro are being actively developed (Vanacker et al., 2012; Luyckx et al., 2013). The main conception of this technology is the encapsulation of ovarian follicles with other ovarian cells into the matrix. At the present time, this technique is at the first stage of its development but it seems to be very promising in the future. These systems will allow better reconstitution of in vivo conditions, resulting in the better stimulation of follicles’ growth and maturation.

Rodent ovarian follicles are most commonly used to develop ovarian tissue in vitro culture techniques. However, it is impossible to create an effective technology for medical practice on the basis of rodent ovarian tissue. Firstly, rodents have shorter ovarian cycles than the human female’s one. Moreover, the ovarian tissue of primates has a higher density than the rodent one. Therefore, for the development of medical technology, studies on primate ovarian tissue in vitro culture are needed.

There is little scientific research related to primate ovarian follicles in vitro culture. However, the obtained results seem to be very promising. It has been shown that baboon follicles can produce mature oocytes that are capable of fertilization and can develop until the morula stage after fertilization via the intracytoplasmic sperm injection (ICSI) procedure (Xu et al., 2011b).

Other research that was performed on rhesus monkeys has demonstrated that more rigid alginate hydrogels allow for better development of follicles than softer ones do (Hornick et al., 2012). Moreover, mature oocytes capable of fertilization and further development up to the morula stage after fertilization by using the ICSI procedure have been obtained during a study on rhesus monkey ovarian follicles, using a fibrin-alginate hydrogel culture system (Xu et al., 2013).

Research studies dedicated to human ovarian follicles in vitro culture are few in number. However, there are very encouraging results among that research. In the early works on human ovarian follicles in vitro culture, follicles were encapsulated into the agar (Roy and Treacy, 1993) or collagen gel (Abir et al. 1997, 1999, 2001), and they maintained their morphology. Moreover, some of the follicles developed until the early antral stages. A recent study has shown that alginate hydrogel permits the growth of encapsulated human ovarian follicles, corresponding to the normal folliculogenesis in vivo. Furthermore, an increase of oestradiol, progesterone and inhibin A during the period of cultivation has been observed (Xu et al., 2009). These hormones are required for normal follicular development.

A scientific group from Belgium has shown that human ovarian follicles can grow in alginate hydrogel. Moreover, in 90% of cases, follicular cells and oocytes retained their viability (Amorim et al., 2009).

In more recent work, researchers from the USA developed an alginate culture system that imitates follicles’ microenvironment in vivo. Moreover, this scientific group (Skory et al., 2015) has developed alginate hydrogel ovarian follicles in vitro culture system that reconstitutes natural conditions. Hormone levels (17β-oestradiol, progesterone, inhibin A, inhibin B, activin A and anti-Müllerian hormone) of cultured follicles were observed by an enzyme-linked immunosorben
assay. When the level of 17β-oestradiol increased and stabilized on the highest value, ovulation was induced by the addition of human chorionic gonadotropin (hCG) and epidermal growth factor (EGF) into the culture medium. Due to the monitoring of follicular hormone secretion, researchers imitated menstrual cycle conditions in vitro by adding hormones and growth factors to the culture medium.

The human ovary contains a large portion of connective tissue, the amount of which varies greatly from patient to patient. Therefore, it appears impossible to develop a standard protocol of a primordial follicle’s isolation from human ovarian tissue. This is due to the fact that the period of enzymatic treatment of human ovarian tissue for the obtaining of primordial follicles varies noticeably in different patients. Moreover, the loss of the integrity of primordial follicles during this procedure, negatively influences their condition (Laronda et al., 2014). Therefore, for better female fertility preservation, both techniques can be used: ovarian tissue cryopreservation in the case of primordial follicles, and ovarian follicle in vitro culture for follicles of advanced stages.

A two-step culture system is another approach that can also be performed for primordial follicle preservation. In these systems, small fragments of ovarian cortical tissue that contain primordial follicles are first cultured in order to stimulate follicular development. After the cultivation, follicles of advanced stages of folliculogenesis (primary, secondary, etc.) are isolated from the ovarian tissue. Subsequent cultivation is carried out with the obtained follicles (Telfer et al., 2008; McLaughlin et al., 2014).

Akt-signalling pathway inactivation allows to stimulate growth of primordial follicles in vitro. The Akt-pathway suppressor is PTEN (phosphatase and tensin homologue). It has been shown that PTEN inactivation results in an increase in the number of growing ovarian follicles in the cases of humans (McLaughlin et al., 2014) and mice (Jagarlamudi et al., 2009).

Nowadays, mature oocytes that are capable of fertilization can be obtained not only from rodents but also from primates. Furthermore, these oocytes can develop after fertilization. Designing human ovarian tissue in vitro culture systems is a very complicated problem. Firstly, it is more difficult to obtain human ovarian tissue for the experiments than in the case of animal models. Secondly, human follicular development in vivo is longer than that of rodents, for example. Due to these facts, human ovarian tissue in vitro culture systems are at the very earliest stages of their development. However, the growth of human ovarian follicles in vitro has still been observed. It is likely that findings that will allow the obtaining of mature human oocytes from ovarian tissue using in vitro culture may appear in the near future.

Conclusion

The first papers dedicated to the cryopreservation of mammalian ovarian tissue were published in 1994 (Aubard et al., 1994; Harp et al., 1994), more than 20 years ago. However, to date, we know of only about a few dozen live births after ovarian tissue orthotopic retransplantation and one live birth after heterotopic retransplantation. In many cases, after ovarian tissue retransplantation, it is impossible to determine whether ovulation occurred in the transplanted tissue or in a remaining fragment of the ovary, especially in the event of natural conception. Therefore, the efficacy of ovarian tissue cryopreservation followed by retransplantation is low. Moreover, the ovarian tissue retransplantation technique cannot be applied in many cases. Replantation of ovarian tissue is an additional surgical procedure that can be dangerous for patients who have undergone chemotherapy or radiotherapy. Furthermore, it is necessary to detect the absence of malignant cells in transplanted tissue. For this reason, several pieces of tissue should be used for analysis that cannot then be transplanted, which reduces the probability of pregnancy. For a time after retransplantation, ovarian tissue is subjected to weak nutrition until angiogenesis is complete. A deficit of nutrients and oxygen leads to apoptosis and atresia in transplanted tissue, which also reduces the probability of pregnancy.

In comparison with the cryopreservation of ovarian tissue followed by retransplantation of thawed fragment ovarian tissue, the in vitro culture technique has a few principal advantages. Firstly, the retransplantation of ovarian tissue is not needed. Secondly, mature oocytes can be obtained and fertilized in vitro, and then embryos can be cryopreserved or transferred into the patient’s uterus or into the womb of a surrogate mother. In the case where ovarian tissue cryopreservation is applied for fertility preservation, surrogate mother programmes require additional procedures such as hormonal stimulation and follicular punctures. These procedures can have a negative effect on patients’ health. An ovarian tissue in vitro culture programme of fertility preservation allows to avoid the risky procedures mentioned above. Moreover, this method permits use of all obtained tissue for the culture. Analysis for the presence of malignant cells in the culture can be performed after tissue culturing. Therefore, the ovarian tissue in vitro culture technique increases the probability of pregnancy when compared with ovarian tissue cryopreservation. The ovarian
### Table 5 Female fertility preservation strategies’ comparison: ovarian tissue in vitro culture vs. cryopreservation

<table>
<thead>
<tr>
<th>Ovarian tissue cryopreservation followed by tissue retransplantation</th>
<th>Ovarian tissue in vitro culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>A second surgical operation is required for the transplantation of thawed tissue</td>
<td>Surgical intervention is needed only for tissue isolation</td>
</tr>
<tr>
<td>Part of the tissue should be analysed for malignant cells’ absence</td>
<td>All obtained tissue can be used for the culture; analysis for the presence of malignant cells can be performed after the tissue culture</td>
</tr>
<tr>
<td>It is difficult to observe each individual follicle</td>
<td>It is easy to observe the growth and development of each individual follicle</td>
</tr>
<tr>
<td>It is difficult to provide good nutrition for the ovary during angiogenesis interruption</td>
<td>Follicles can be cultured individually, facilitating the supply of follicles with nutrients</td>
</tr>
<tr>
<td>Technologies for ovarian tissue cryopreservation have low efficacies</td>
<td>Ovarian tissue in vitro culture allows to obtain mature oocytes capable of fertilization. Oocyte and embryo cryopreservation technologies are more effective in comparison with the cryopreservation of ovarian tissue</td>
</tr>
<tr>
<td>Age limitations – less than 35 years old</td>
<td>No age limitations</td>
</tr>
</tbody>
</table>

The ovarian tissue in vitro culture method allows observation of each follicle individually, and the effective provision of them with necessary nutrients.

Although the ovarian tissue in vitro culture is not currently a well developed technique that is applied widely in clinical practice, there are many results showing the efficacy of this method (Xu et al., 2011a; Jin et al., 2010; Xu et al., 2006b; Choi et al., 2014; Xu et al., 2013; Shikanov et al., 2011; Shikanov et al., 2009). The ovarian tissue in vitro culture technique allows to obtain mature oocytes capable of fertilization and further development, not only in the case of rodents (Xu et al., 2006b), but also for primates (Xu et al., 2011b; Xu et al., 2013). Moreover, the growth of human follicles was observed when using this method (Xu et al., 2009).

Therefore, the ovarian tissue in vitro culture technique seems more promising than ovarian tissue cryopreservation followed by thawed tissue retransplantation. The principal advantages and drawbacks of the methods mentioned above are listed in Table 5. In contrast with cryopreservation, the ovarian tissue in vitro culture technique allows the avoidance of secondary surgical intervention and patient hormonal stimulation. Moreover, in the case of the ovarian tissue in vitro culture technique, it is easier to provide better nutrition for follicles and to observe their growth and development. All of these advantages help practitioners to yield better results.

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### Statement of interest

None.

### References


Female fertility preservation strategies


Female fertility preservation strategies


Female fertility preservation strategies


