Female Fertility Preservation

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1. Abstract

The increasing incidence of malignant diseases that often require gonadotoxic treatment and the tendency to become parent later, results in an increased need for fertility preservation.

The following review summarizes different options for fertility preservation addressed to pre-puberal girls and women at reproductive ages whose fertility has been compromised. They include embryo, unfertilized oocytes and ovarian tissue cryopreservation.

The review also consider new improvements that are being studied for these procedures and recent advances in this field which can open a door to novel treatments for human infertility and fertility preservation.

2. Materials and Methods

✓Scientific literature search on PubMed based on specific words such as: fertility preservation, oocyte and embryo cryopreservation and so on. The research has been focused on recent papers and reviews.

✓Assisted in a conference organized by Víctor Grífols and Lucas Foundation about the 30th anniversary of the first child born in Spain after IVF.

3. Introduction

•**Female fertility** is based on a pool of non-growing follicles in the ovary, some of which start growing every day. This procedure becomes gonadotrophin dependent and ends with ovulation.

•Female fertility preservation has become an **interesting matter** for society in the last time. This interest has grown due to the **increasing rates of cancer** in young population and the **delay in childbearing** for social issues (*following diagram*). Cancer treatments (in pre-puberal and fertile women) cause follicle loss which implies an alteration of reproductive potential.

Necessities of female fertility preservation

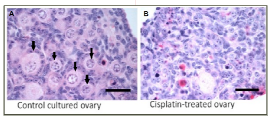


Fig 1: *Illustration of the decline in primordial follicle number due to Cisplatin, a chemotherapeutic agent.* ***A)*** *control cultured mouse ovary, arrows indicate primordial follicles,* ***B)*** *mouse ovary after Cisplatin treatment [1].*

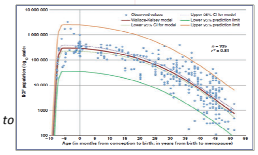


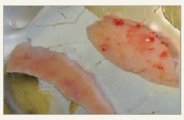
Fig 2. *Decline of nongrowing follicles in the ovary as function of time [2].*

4.Options to Preserve Female Fertility

Ovarian tissue cryopreservation

• **Procedure:** remove ovarian **grafts** (fig. 3) and freeze them. When they are frozen, tissues can be stored in order to use them in the future. Lately, ovarian strips can be thawed and grafted to **orthotopic** or **heterotopic** site in the body. Strips have to restore ovarian endocrine function permeating oocyte maturation. Pregnancies after transplantation can occur by natural conception or after IVF techniques.
• **Indications**: addressed to pre-puberal girls who have to be subjected in invasive treatments.
• **Advantages**:1) No ovarian stimulation is need, 2) All ages, 3) no male is need.
• **Disadvantages**: 1) requires surgery, 2) risk of reimplant malignant cells.

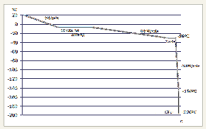
Fig 3*: Large ovarian cortical strips prepared for transplantation. They have been thawed after having been stored in low temperatures [3].*



Slow freezing

• **Procedure:** Used for **embryo cryopreservation**. Slow freezing protocols consist in cooling the samples by cooling rates (fig. 4). In order to prevent intracellular ice formation and toxic concentrations of solutes the freezing solution must be supplemented with cryoprotective additives (CPA).Once embryos are in LN_2 they can be stored for a long time.
• **Indications:** addressed to women in reproductive age who want to delay motherhood or women who have to be submitted on invasive treatment.
• **Advantages**:1) No surgical procedure, 2) No risk of reimplant malignant cells, 3) long time storage.
• **Disadvantages:** 1) Ovarian stimulation is need, 2) Not indicated for pre-puberal girls, 3) male is need. 4) embryo→ ethical concerns, 5) Requires IVF.

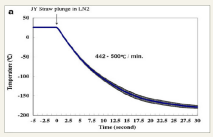
Fig 4: *Slow freezing cooling rates. Lowering temperature is made in different times At 7ºC ice seeding is induced. Then cooling procedure continues since LN_2.*



Vitrification

• **Procedure:** used for both **embryo and oocyte cryopreservation**. Consist in super rapid cooling procedure (Fig. 5) achieved by putting the samples in direct contact with LN_2. The aim of vitrification is to prevent ice formation and too fast dehydration oocyte/embryo, that's the reason why concentrations of CPA are extremely high.
• **Indications:** addressed to women in reproductive age who want to delay motherhood or women who have to be submitted on invasive treatment.
• **Advantages**:1) No surgical procedure, 2) No risk of reimplant malignant cells, 3) long time storage, 4) oocyte→less ethical concerns.
• **Disadvantages:** 1) Ovarian stimulation is need, 2) Not indicated for pre-puberal girls, 3) male is need. 4) embryo→ethical concerns, 5) Requires IVF.

Fig 5: *Typical vitrification cooling curve. Samples are introduced in LN_2 without using cooling rates. The procedure is really fast [4].*



5. Future Goals

The future of female fertility preservation comes to us by two different ways:

1. Groups who want to **improve and optimize procedures** and techniques used nowadays in clinic practices: ovarian tissue, embryo and oocyte cryopreservation.
2. Researches interested in looking for new strategies far from cryopreservation. **Stem cells-based strategies** (table) for ovarian regeneration and oocyte production has been proposed as future clinical therapies for female infertility.

Table*: Characteristics of stem cells used in stem cell-based therapy research of infertility.*

ESCs	MSCs	Stem cell from extraembryonic tissues	IPSCs	OSCs
Derived from inner cell mass of the blastocyst.	Derived from bone marrow, adipose tissues, bone, Warton's jelly, umbilical cord blood and peripheral blood.	Derived from amnion, chorion, placenta and umbilical cord.	Derived from somatic cells.	Derived from ovarian tissue.
Pluripotent	**Multipotent**	**Multipotent**	**Pluripotent**	**Multipotent**
Prolonged proliferation.	Degree of proliferation depends on the tissue from which these cells were isolated.	Degree of proliferation depends on the tissue from which these cells were isolated.	Prolonged proliferation.	Uncertain proliferation in the ovary. Achieved in vitro.
Indefinite self-renewal potential.	Limited self-renewal.	Limited self-renewal.	Indefinite self-renewal potential.	Unknown.
Immortal; cell lines remain intact for long periods of time.	Production of limited number of cells.	Production of limited number of cells.	Immortal; cell lines remain intact for long periods of time.	Production of limited number of cells.
Ethical concerns.	**Less ethical concerns.**	**Less ethical concerns.**	**Less ethical concerns.**	**Less ethical concerns.**

6. Conclusions

✓The **need for female fertility preservation is emerging** because the incidence of cancer in young population is increasing and its treatment usually leads to infertility. Fertility preservation is also needed because many women prefer to postpone childbearing.

✓Nowadays there are some **options available** in order to preserve female fertility, most common are: **ovarian tissue, oocyte and embryo cryopreservation**. These techniques consist in freezing the samples following different protocols according to their characteristics and storing them in LN_2 for future applications.

✓**Not all procedures can be used for all patients**: while ovarian tissue cryopreservation is more suitable for pre-puberal girls, oocyte and embryo cryopreservation is the main election for women at reproductive ages.

✓Protocols about samples cryopreservation have been designed to prevent as much as possible cell alterations and death. **Survival rates for slow frozen oocytes are much lower than vitrified ones**, that's the reason why slow freezing for oocytes has not been used anymore in clinics.

✓Future goals on female fertility preservation are the **improvements** and **optimization** of current procedures.

✓There are some researchers looking for new strategies, they believe that an ideal fertility preservation approach would prevent delays in commencing live-saving treatment and avoid transplanting malignant cells back . **Stem cells strategies** (in particular OSCs) may offer one route to achieve this goal.

7. References

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