ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Master in:

"MINIMALLY INVASIVE AND ROBOTIC PEDIATRIC SURGERY"

A.Y. 2020/2021

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Thesis Title

Ovarian tissue collection for fertility preservation in children with malignancies. Twenty-years experience of two high-volume Centers: follow-up and technical considerations.

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Introduction

Survival after childhood cancer has improved during the last two decades and is now up to 80% considering all diseases, and nearly 75% of the patients will be living more than 10 years after diagnosis (1).

Even more, long-term survival rate of children undergoing haematopoietic stem cell transplant (HSCT) is constantly increasing.

It is now well known that improving in survival presents, on the other side, an increase in mortality and morbidity in long term survivors.

Among all the late effects, infertility is reported as a major concern, especially in female cancer survivors.

Cancer treatment often involves aggressive radiotherapy or chemotherapy, which may permanently impair reproductive function.

In particular, total body irradiation (TBI) and older age at time of HSCT can negatively affect the persistence of ovarian function and the onset of premature ovarian failure (POF).

When administered before puberty, TBI is less gonadotoxic, with 40-60% of patients experiencing spontaneous recovery versus 10-14% in post-pubertal girls.

The protective effect of younger age might be related to the higher number of nongrowing follicles, to the higher resistance of primordial follicles to vascular phenomena and fibrosis or to paracrine factors.

Moreover, a model has been evaluated to predict the age of onset of menopause according to radiation dose and age at irradiation.

Loss of ovarian function after chemotherapy that includes an alkylating agent (cyclophosphamide, busulfan) could result in both sterilization and endocrine function deficiency as ovarian hormonal production is closely related to the presence of oocytes and maturation of the primary follicles.

Due to all these factors the risk of infertility in patients undergoing conditioning regimen for HSCT has been defined as >80%.

Fertility preservation is a key component of POF management in young people and should be considered for all young people undergoing potentially gonadotoxic cancer treatments or at high risk for ovarian failure.

Cryopreservation of ovarian tissue is the main option available to preserve fertility in women who require cancer treatment but cannot delay the chemotherapy and in prepubertal patients.

The advantage is that it requires just few days to plan and perform the laparoscopic surgery and, as the retrieval of ovarian tissue is not dependent on the menstrual cycle, no delay in treatments is required.

Moreover, this technique allows the storage of a great number of primordial follicles that are relatively resistant to cryodamage (about 70%–80% survival).

We compared the experience of two high-volume pediatric Centers in the north of Italy, where, since 2000, female patients at high risk for subsequent infertility, are enrolled in the program of laparoscopic ovarian tissue cryopreservation.

Material and Methods

Two high-volume pediatric Centers were considered for this study: the Regina Margherita Children's Hospital, part of the University Hospital of Health and Science of Turin (Center 1) and the IRCCS Sant' Orsola-Malpighi University Hospital in Bologna (Center 2).

This is a retrospective study including girls and adolescents up to 17 years of age who underwent ovarian tissue cryopreservation in the last two decades, before a highly gonadotoxic treatment for malignant or non-malignant (hematologic) diseases was initiated.

The indication of ovarian cryopreservation was established when the treatment planned included conditioning for autologous or allogeneic hematologic stem cell transplantation, or high-dose chemotherapy, in toto abdominal irradiation or pelvic irradiation.

Patients in whom fertility may be prematurely altered, as in case of Turner syndrome, were excluded from this study.

Results

Center 1

The experience in Center 1 in laparoscopic ovarian tissue sampling for cryopreservation in children (< 18 years) affected by haematologic, immunologic or neoplastic diseases, was considered.

A total of 105 procedures were performed in patients undergoing HSCT or sterilizing chemotherapy.

The median age at diagnosis was 11.12 years (range: 0-17.49 years).

The median age at the time of procedure was 13 years (range: 2.7-20.3 years).

24 patients were not pubertal at the time of surgery.

A 3-trocars (5mm) laparoscopy was performed, collecting 50% of tissue of each gonad (ovarian cortex biopsies) without tissue cauterization to optimize the viability, and without draining the pelvis [Figure 1].

The tissue was then submitted to histologic examination to detect any tumour contamination and then frozen following the slow freezing procedure and cryopreserved in liquid nitrogen.

Trocar position was the following: the 0 degree camera port in the navel and the two operative ports in the right and left iliac fossa respectively (Figure 2).

Mean operative time was 45 minutes.

Hemoglobin check was planned in the first post-operative day.

Any early or late complications (nor bleeding, nor reoperation) was registered and no drain was left in place.

No malignant cells were identified at the histopathology evaluation and average time of discharge was 48 hours after surgery.

Primary pathologies of the patients were:

- acute leukemia (n 60)
- β-thalassemia (n 12)
- Hodgkin's lymphoma (n 10)
- non-Hodgkin's lymphoma (n 10)
- others (n 13)

Currently in Center 1, a 29-year-old patient affected by β-thalassemia had a successful pregnancy after autologous orthotopic transplantation of cryopreserved ovarian cortical tissue, while, to date, 30 live births have been reported worldwide.

Center 2

In Center 2, a total of 207 patients were treated from January 2002 to October 2021.

Mean age was 13 years old (± 4,19 aa, range 1,8- 17,9), 42 patients were not pubertal at the time of surgery.

A pelvic ultrasound was performed in the pre-operative period to evaluate ovarian morphology and laboratory tests that includes hormonal assays and virological screening.

The technique involved the use of 3 trocars, one 10 mm umbilical port for 0 degree camera and two operating trocars preferably 5mm to have a more stable grip, and hemostatic devices.

The mean operating time was of 40 minutes.

Only one ovary was subjected to biopsy, based on the pre-operative pelvic ultrasound.

Surgical steps were: the stabilization of the ovary by means of grasping forceps, followed by cutting the tissue to be removed with a cold blade.

The tissue was excised with atraumatic forceps in a single point.

Then the piece was externalized from the navel and accurate hemostasis was carried out with a monopolar hook or by means of bipolar forceps.

The main goals of the procedure were: the limitation of bleeding and for this reason a slight pressure on the hilum was useful, using a cold blade to avoid thermal damage, limiting the manipulation of the tissue to be removed and avoid tubal trauma. Hemostasis did not require special tools.

Sometimes argon was used because of its action limited to the surface layers.

At the end of the procedure, a drain was left in place and removed in the first postoperative day, after performing a control blood count.
The discharge took place in the same afternoon or on the first or second post-operative day.
The neoplastic pathology was predominant (77%), followed by hematologic conditions:
- leukemia/lymphoma (n93)
- sarcomas (n47)
- central nervous system tumors (n10)
- others malignancies (n8)
- Wilms' tumor (n6)
- Talassemia (n16)
- hematologic conditions (n20)
- others (Turner disease, etc.) (n7)

The median age was 13 years and 3 months and the range was from just under 2 years to almost 18 years. There were 42 prepubertal patients.
Over time the technique has undergone some changes.
Initially the ovary was sutured for hemostatic purposes, but currently the slice is just coagulated.
In the first cases the piece was inserted into a glove finger, in order not to damage it, but sometimes the maneuver could be demanding, and the tissue could be hammered during the manipulation and therefore now is simply extracted from the navel.
The only other preservation technique applied in prepubertal age was ovarian transposition. However, this was reserved for patients who need radiotherapy and was performed in this series in 8 cases.
Another variant concerned the removed tissue.
Until a few years ago the ovary was sampled bilaterally in order to obtain a sufficient amount of tissue.
They currently tend to take a large part of one ovary only.

Cryopreservation/Pathologic evaluation

The ovarian cortex is located just deep to the capsule and is where the majority of the primordial follicles reside. These primordial follicles represent the child's ovarian reserve.
In the tissue cryopreservation technique the cortex is dissected from the medulla and cut into cortical strips, washed to remove blood cells, and then passed through a series of cryopreservation media.
The processed strips are placed in cryovials and undergo a slow-freeze process to −40 °C.

The tissues are then transported to a long-term reproductive tissue storage facility in liquid nitrogen. The intraoperative biopsy undergoes pathology evaluation for the presence and stage of ovarian follicles, and the eventual presence of malignancy.

If evidence of gross solid tumor is found within the ovary, the cryopreserved tissue is recalled for further pathologic staging and is not stored for the patient's use.

For patients with acute lymphoblastic leukemia or lymphoma, the ovarian stroma may contain malignant cells.

Since the ovarian tissue is not required for further pathologic staging, the tissue may be cryopreserved for the patient's use with the understanding that it cannot be transplanted back into the patient. However, future fertility restoration may be possible through follicle isolation, in vitro follicle maturation, and in vitro fertilization.

Surgical techniques for ovarian tissue transplantation

There are a number of different surgical approaches for transplanting cryopreserved ovarian tissue (Ovarian Tissue Transplantation – OTT) [Figure 3,4,5].

The choice between orthotopic or heterotopic graft sites depend on patient disease factors as well as any adjuvant therapies such as removal of ovaries or pelvic irradiation.

Various surgical techniques such as laparoscopy or robotic surgery and use of allografts such as Alloderm have also been trialled in an attempt to increase the success of follicular stimulation from OTT, but ongoing experimentation is likely to be required (8).

A graft needs to be securely fixed to its recipient bed to reduce bleeding, infection and shearing forces and promote neoangiogenesis to allow the graft to take.

Heterotopic OTT in superficial sites may be especially prone to graft movement but it is also important when fixing tissue within the peritoneal cavity.

Fixation of tissue in OTT varies significantly, depending on graft location and surgeon preference. Transplantation of tissue fragments onto a denuded ovary have been attached using sutures (such as 7/0 or 8/0 polypropylene or 9/0 nylon), intercede and/or fibrin glue.

They may also be placed in either subcortical pockets in the ovary or peritoneal pockets, with the incisions closed if required with either fibrin glue, staples or sutures (e.g., 4/0 vicryl) to hold them in place.

It should be noted, however, that these interventions minimize graft mobility but may increase risk of haematoma or seroma formation due to impaired drainage, and tissues should be handled as little as possible to prevent mechanical damage, especially when fragments are small.

Skin grafts are also immobilised using various techniques, including sutures, staples or fibrin glue, influenced by location of the graft site, age of the patient and indication for the graft.
The main difference is that skin grafts are superficial and can often overlie an area of high mobility, e.g., muscle, and patients are often required to rest in bed for a number of days to allow for maximum chance of graft take.

Discussion

This study report of a large multicentric series of young patients including girls and adolescents younger than 17 years of age, that underwent fertility preservation through OTC at two high-volume Pediatric Centers.
The patients in our study had a high proportion of pediatric-specific diseases such as neuroblastoma but also common childhood diseases such as leukemia and central nervous system tumors.
Apart from leukemia, the most common pathologies found were bone tumors and lymphoma.
As the surgical approaches, there are some differences between the two Centers.
Bilateral harvesting in Center 1 is opposed to unilateral biopsy in Center 2, adding the pre-operative ultrasound evaluation.
The drain was left in place only in Center 2, followed by hemoglobin check in the first post-operative day in both Centers.
The trocar position was similar but others configurations are possible (Figure).
No minor o major complications were reported in the two Institutions.
To date, transplantation of cryopreserved ovarian tissue has resulted in births of at least 130 children but data on transplantation of ovarian tissue removed before puberty are scarce.
Currently in the literature, 4 patients under 15 years of age at the time of OTC have used their cryopreserved ovarian tissue (2).
The first publication showing the functionality of ovarian tissue cryopreserved before puberty was published in 2012. This was a patient with sickle cell disease in whom ovarian freezing was carried out at age 10 before a myeloablative conditioning regimen followed by allogeneic HSCT.
Twenty-seven months after the HSCT, the patient and her mother returned to request an ovarian tissue autotransplantation to induce spontaneous puberty because she had premature ovarian failure.
A heterotopic autotransplantation of three fragments of ovarian cortex succeeding in inducing puberty with the onset of the first menstrual period 8 months after the ovarian transplantation (3).
The following year, Ernst et al, confirmed that ovarian cortex transplantation could induce puberty in a patient who was 9 years old at the time of OTC prior to treatment for Ewing's sarcoma (4).

The first birth obtained after transplantation of ovarian tissue cryopreserved before menarche was reported in 2015 (5).

This patient underwent OTC before HSCT for the treatment of sickle cell disease.

At the time of the ovarian cryopreservation she was almost 14 years old and had not had any periods yet.

Ten years later, the patient had a premature ovarian failure and requested an ovarian tissue transplantation to restore her fertility.

She had her first menstrual period 5 months after the ovarian transplantation and became spontaneously pregnant more than 2 years after the transplantation, giving birth to a healthy boy.

The youngest patient at the time of ovarian freezing, who gave birth after ovarian cortex transplantation, was 9 years old at the time of tissue retrieval.

She had beta-thalassemia treated by HSCT preceded by gonadotoxic conditioning regimen before HSCT (6).

These rare publications confirm that beyond the age of 9, ovarian tissue can be functional after transplantation (7).

It remains to be determined whether this can be applied for girls younger than 9 years old.

Regarding ethical and medico-legal issues, ideally, a fertility preservation technique should be: evidence-based, do not create false hopes or unrealistic expectations and do not harm.

The problem with cryopreservation is the experimental label which has not yet been removed in many countries, especially because the evaluation of effectiveness takes many years and because of the risk of reimplantation of cancer cells.

If these factors on the one hand can create some doubts, on the other we must consider that many studies report distress, anxiety and depression related to future infertility and repentance for not having seized the possibilities and it is undeniable that fertility belongs to those factors that are enclosed in the broader definition of individual health, therefore we are obliged to take care of it.

Cryopreservation is the only weapon pediatric surgeons have in prepubertal patients, so we don't have so much choice, the only alternative is to do nothing, which perhaps would be less ethical.

In addition, this procedure has not caused severe complications, there are very few cases and certainly we perform much more risky procedures.

We must also consider the positive effect that thinking about the future provides to patients in dealing with the underlying pathology.

Finally, the advantage of the pediatric age is precisely time, that is, the fact that we do not know how many progresses science will make when this tissue will be requested by the patient in 10 or 20 years and we may be able to offer her new therapeutic possibilities.

Conclusions

Ovarian tissue cryopreservation is a promising modality to preserve fertility.

The technique is safe, easy to perform and reliable and offers a minimal invasive approach in high-risk patients, without delaying the onset of chemo or radiotherapy.

The series of both Centers were comparable in terms of technical approaches and outcomes.

A team of specialized surgeons is needed to grant a standardized procedure.

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Figures

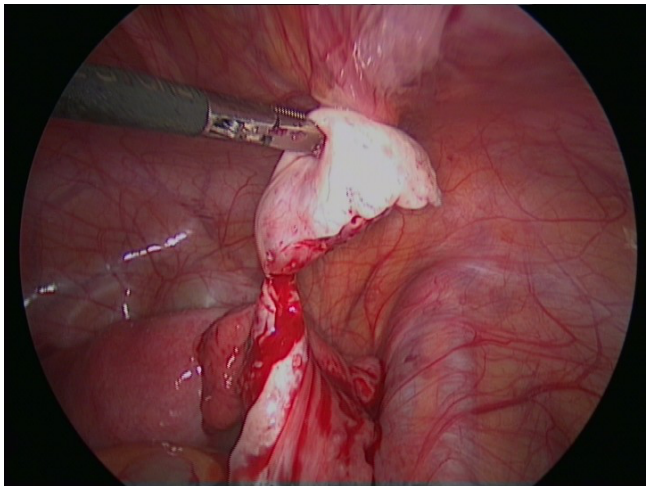

Figure 1. Intraoperative view of the ovarian biopsy (Courtesy: Michela Maffi, MD)

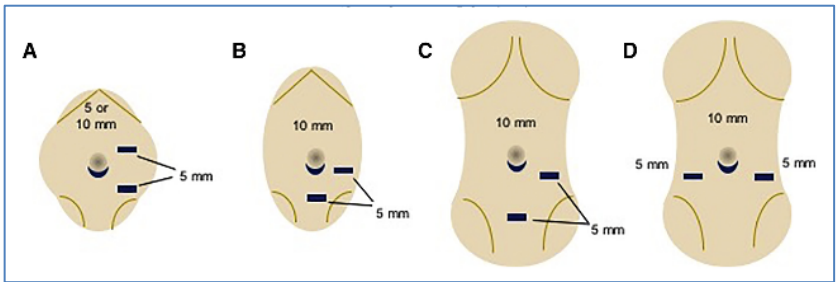

Figure 2: option for trocar position.

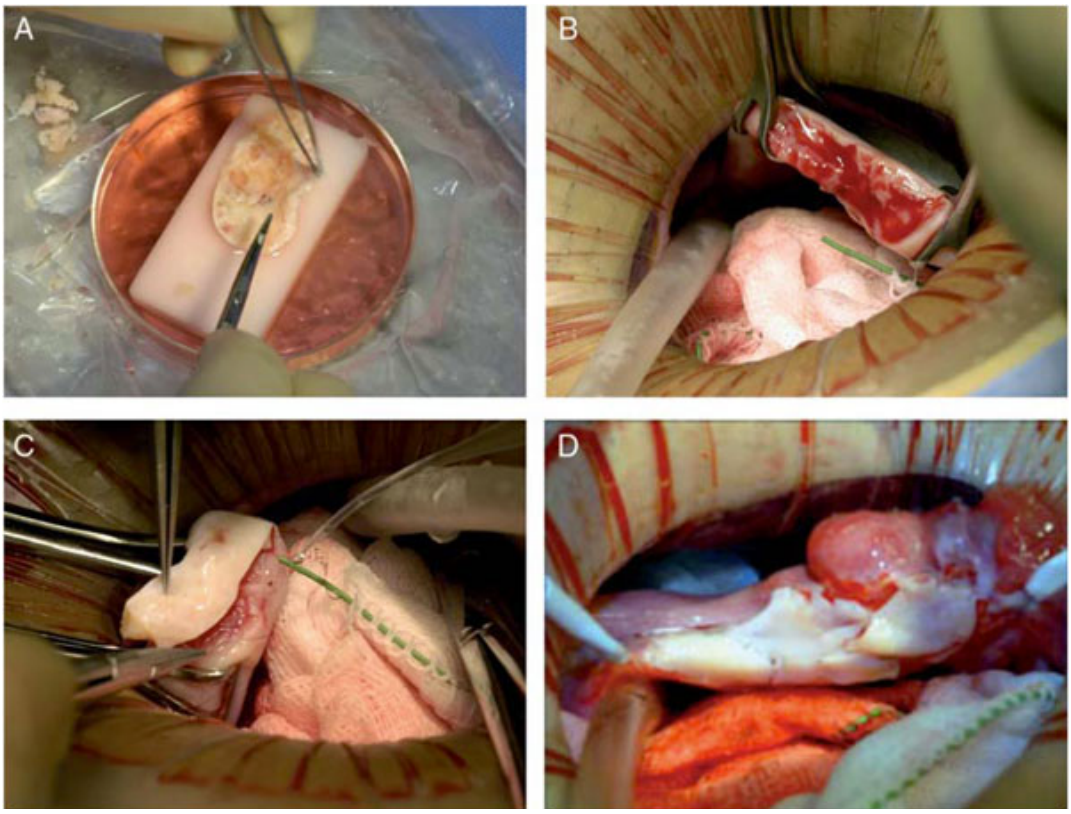

Figure 3: the open transplantation technique.

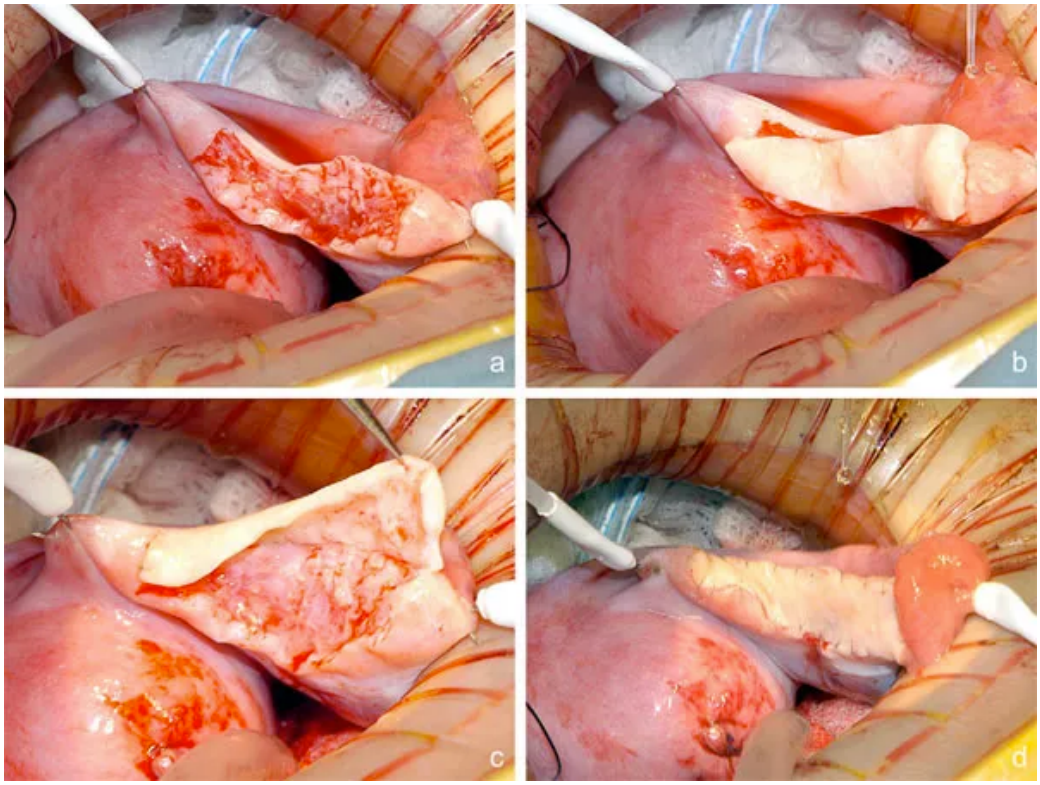

Figure 4: more detailed view of the transplantation procedure.

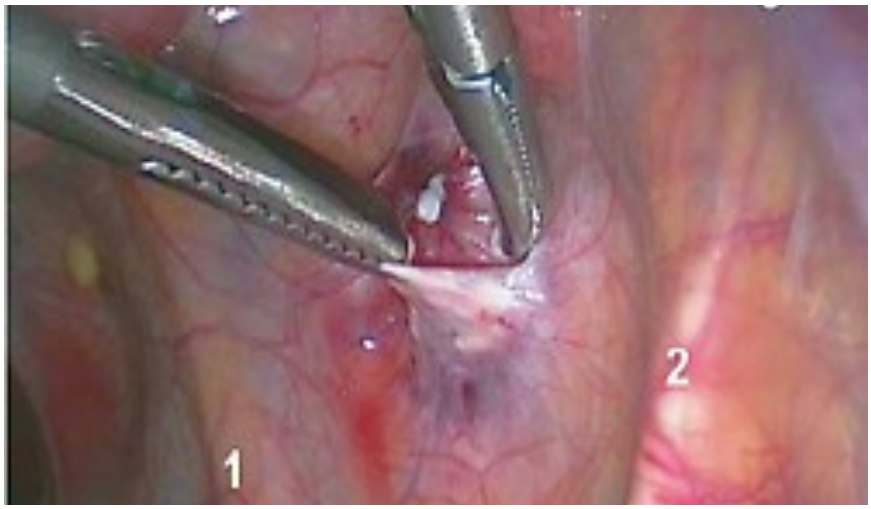

Figure 5: laparoscopic transplantation technique.

Acknowledgments

Prof. Mario Lima

For the opportunity to share with me his great experience, and his kindness.

Prof. Tommaso Gargano

For his continuous support, patience, friendship, and perfect course organization.

Dr. Michela Maffi

Without her support and her data shared with me, this thesis would not have existed.

The whole equipe of pediatric surgeons of Sant'Orsola Hospital

Thanks for their professionality and for always welcoming me with a smile.

Dr. Alessandro Pane

My dear friend and precious colleague in Turin, for the beautiful time spent in Bologna.