Bratisl Lek Listy 2010; 111 (8)
443 – 448

CLINICAL STUDY

Protection of ovarian tissue from radiotherapy

Pafumi C[1], Iraci SM[2], Abbate G[1], Clemente CM[2], La Rosa I[1], Gulino FA[4], Iemmola A[4], Randazzo C[1], Caldaci L[4], Teodoro MC[4], Palumbo MA[4], Attard M[3], Ciotta L[5], Pagano I[5], Stracquadanio MG[5], Messina A[1]

Department of Obstetrics and Gynaecology, University of Catania, Italy. **pafumi@unict.it**

Abstract: Advances researches in the diagnosis and treatment of childhood, adolescent and adult cancer have greatly increased the life expectancy of premenopausal women with cancer. However, one of the serious side effects of these treatments is the risk of damage to fertility. The ovaries are very sensitive to cytotoxic and radiotherapeutic treatment. The only established method of fertility preservation is embryo cryopreservation according to the Ethics Committee of the American Society for Reproductive Medicine (2005), but this option requires the patient to be of pubertal age, have a partner or use donor sperm, and be able to undergo a cycle of ovarian stimulation, which is not possible when the radiotherapy has to be initiated immediately or when stimulation is contraindicated according to the type of cancer. For patients who need immediate radiotherapy, cryopreservation of ovarian tissue is the only possible alternative. This manuscript reports the different techniques of cryopreservation and the results of transplantation of cryopreserved ovarian tissue. The current techniques allow cryopreservation of human ovarian fragments for a long time with good follicular survival rate after thawing. Numerous studies ultimately in this field have demonstrated to improve the survival rate of the oocytes and cryopreserved follicles. Moreover this manuscript includes a case of a 17-year-old girl who had to undergo pelvic irradiation for non-Hodgkin's lymphoma and the laparoscopic treatment to preserve the fertility (Fig. 2, Ref. 47). Full Text in free PDF *www.bmj.sk.*
Key words: ovarian tissue, cancer, laparoscopic treatment, radiotherapy.

The first cryopreservation experiments on ovarian tissue were performed in the 1950s–60s, when fragments of ovarian walls were frozen at a temperature of -79 °C in a solution containing glycerol (1, 2, 3).

The first pregnancy using cryopreserved and subsequently thawed ovarian tissue was obtained in mice as early as 1960 (4). However, the last fifteen years gave raise to the creation of innovative protocols of freezing and thawing which have helped achieve encouraging results in animals, thus opening the door to experimentation on human subjects.

Today, cryopreservation of ovarian tissue represents one of the most ambitious strategies in the attempt to preserve fertility of women with pathologies, mostly malignant neoplasms which can compromise the ovarian follicle reservoir and reproductive potential.

Cryopreservation of ovarian fragments is applicable even to patients in pediatric cohort. It does not assume any hormonal stimulation to the ovary. Ovarian biopsy allows to obtain hundreds of primordial follicles containing immature oocytes, which are very resistant to the processes of freezing and thawing (5).

[1]Department of Obstetrics and Gynaecology, University of Catania, Italy, [2]Department of Endoscopic Gynaecology S. Bambino Hospital, Catania, Italy, [3]Faculty of Medicine & Surgery, University of Malta, [4]Department of Assisted Reproduction, Faculty of Medicine, University of Catania, Italy, and [5]Department of Gynaecological Endocrinology

Address for correspondence: C. Pafumi, Prof., Department of Obstetrics and Gynaecology, University of Catania, Italy

Indications to cryopreservation of human ovarian fragments

Benefits of ovarian fragments cryopreservation may be found in the following situations:

– *Patients affected by neoplasms:* in recent years, anti-tumor strategies have brought a progressive increase in the survival rate of girls and young women affected by neoplasms like lymphomas, leukemia, breast tumors and sarcomas, directing attention to the long-term effects of counter cancer therapies and the quality of life of the patients afterwards. It has been estimated that at the beginning of the 21st century, 1 adult in every 1000 who had had a tumor during the pediatric phase of their life, will survive until the third decade of their life.

In tissues with rapid cellular proliferation, such as bone marrow and intestines, the cytotoxic damage inflicted by chemotherapy and radiotherapy is almost fully reversible. At the ovarian level, where the number of germinal cells is limited and pre-determined at birth, the damage is usually progressive and irreversible. In case of a substantial percentage of patients, chemo- and radio-therapeutic treatments are capable of causing temporary or persistent oligo/amenorrhea, and in the long-term they may lead to premature menopause. Such therapies, moreover, are capable of damaging, in an unpredictable way, the genetic integrity of the oocytes. There are numerous causes of the damage to the reproductive potential and they depend on the age of the patient, type of treatments assigned by oncologists and the initial size of the ovarian follicle reservoir (6–15).

On a psychological level, the prospective loss of the reproductive potential increases the stress of the patients, who are already concerned with the risk of a possibly long-term deterioration of the quality of their life. Presenting the possibility of losing fertility and emphasizing the fact that there are possible solutions to the problem, at the time of communicating the oncological diagnosis, offers an increased hope of a cancer-free future, in which life should return to normal as much as possible (16).

– *Risk of bilateral oophorectomy* as a treatment for benign ovarian cysts, for example in recurring endometriosis or in case of benign bilateral teratomas.
– *Family history of premature ovarian failure* in patients whose mothers reached menopause before 42 years of age and who thus carry an increased risk of premature loss of ovarian function, which can also occur at a young age (even before 30).

Cryopreservation of ovarian tissue

Research in the field of cryopreservation of ovarian tissue has gained momentum only in the recent years, when Gosden resumed his study in this area, while also adopting more modern freezing techniques (17, 18).

Protocols of slow freezing/rapid thawing of ovarian fragments, as well as of the ovary as a whole, have been applied in mice, rats and sheep, utilizing dimethyl sulfoxide (DMSO) or propane-1,2-diol as cryoprotectants. With these techniques, survival rate of primordial follicles to thawing, which in the past was very low, has reached approximately 70–80 %. It allowed to restore and maintain ovarian function in animals after autologous transplantation of thawed fragments for a number of months. Although there is evident damage of ischemia, related to the impossibility of reanastamosing vascular supply, histological analysis performed on transplanted fragments showed evidence of a good follicular survival ratio, even after a period of some weeks (19, 20, 21). Spontaneous pregnancies have been obtained after autologous transplantation of cryopreserved ovarian fragments in rats (22), in sheep (23, 24) and recently even in women (45).

In 1996, Hovatta et al (25) carried out the first freezing experiment on human ovarian tissue, demonstrating that human ovary is resistant to the cryopreservation procedures. Successive studies have confirmed that in human ovarian tissue, the primordial follicles are the most resistant ones and are able to resist the damage/s induced by freezing/thawing procedures. These damages are related, above all, to ischemia during the first post-transplantation hours, preceding spontaneous revascularization of the tissue (26, 27). Recently, the presence of vital primordial follicles, found in human ovarian tissue and transplanted in immunodeficient mice (SCID mice), has been demonstrated, together with the possibility of inducing ovulation of the xenotransplant using exogenous follicle-stimulating hormone (FSH) (28–30).

Techniques of obtaining ovarian fragments

Ovarian tissue destined for cryopreservation is normally withdrawn during the course of a laparoscopic procedure, unless the patient has to undergo a laparotomy due to other indications. The laparoscopic procedure presents notable advantages:

– the possibility of it being carried out with minimal previous warning, thus avoiding postponement of any type of oncological therapy;
– very low percentage of operative and post-operative complications;
– brief duration of the procedure (15–20 minutes);
– absence of protocols to follow after the completion of the procedure.

Considering that 35 primordial follicles can be counted per square millimeter of ovarian cortex in a woman of about 30 years of age, five cube-shaped cortical fragments of 5mm sides are sufficient to obtain more than 4,000 primordial follicles.

The fragments can be withdrawn by superficial cutting of the ovarian cortex with laparoscopic scissors or a specific metal instrument devised for biopsies in order to obtain fragments of 5 mm in diameter and 2–3 mm of thickness (31).

In the event when ovariectomy has to be performed, it is often possible to find and cryopreserve healthy fragments of ovarian parenchyma at the borders of the part to be excised.

Withdrawal of the biopsied fragments of the ovarian cortex is applicable also to pediatric patients and contextually even for other surgical procedures (e.g. aspiration of bone marrow). It represents a justified approach even in patients in whom there is a probability of spontaneous recovery of ovarian function post-radio/chemotherapy. In this case, it offers these patients a sort of „double possibility“ of preserving part of their reproductive potential.

More radical procedures, like monolateral ovariectomy, can be taken into consideration in patients who will undergo sterilizing treatments (e.g. total body radiotherapy) by cryopreserving the whole ovarian cortex.

If a patient is treated exclusively with pelvic radiotherapy (e.g. for cancer of the uterine cervix), it is also possible to temporarily autotransplant the whole ovary into another location (e.g. into a pocket between the muscles of the arm), following a vascular microanastomosis. On completion of the radiotherapeutic treatment, the ovary could be placed back in its original position, anastamosing the ovarian blood vessels. One should be wary that ovarian metastasis have been reported in patients with cervical cancer, however, ovarian cancer rate following radiotherapy is slim (46).

Technique of freezing and thawing human ovarian tissue

The major part of the currently used protocols require slow freezing and rapid thawing. Ovarian fragments, placed in a freezing solution in the cryotubes, are gradually frozen in a programmable freezer and subsequently immersed in liquid nitrogen, where they can be preserved for an indeterminate amount of time (26).

Furthermore, techniques of rapid freezing in the presence of elevated concentrations of cryopreservants (vitrification) are being studied, and have already been applied successfully to the cryopreservation of human ovarian tissue (32).

At thawing survival rate of primordial follicles in the human ovary varies, depending on cryopreservants: 84 % for ethane-1,2-diol, 74 % and 44 % for dimethyl sulfoxide and propane-1,2-diol respectively, with only 10% for glycerol (26). Other non-permeating cryopreservants, such as saccharose and mannitol, are usually added to the solution in association with proteinaceous substances derived from serum or synthetic, and which play a protective role for the plasma membrane of the oocyte in the final phase of thawing.

Autotransplantation of ovarian tissue after thawing

The most complex problem to deal with is the survival of only primordial follicles to thawing, which contain immature, diploid oocytes and which are completely inept for fertilization. The maturation of these follicles (and oocytes contained within) is an essential requisite to achieve pregnancy.

There are different ways of achieving this. Currently the most promising one seems to be autotransplantation. Cryopreserved ovarian fragments are positioned in their original location (ovarian fossa) by means of autotransplantation via laparoscopy. Oktay et al. have demonstrated the possibility of rooting transplanted fragments and inducing ovulation using gonadotropin in a period from 15 weeks up to 6 months from the autotransplant (33, 34). This technique, devised to resume ovulation from the original position of the ovary, theoretically offers the possibility of obtaining spontaneous conception without having to use *in vitro* fertilization techniques. However, since the ovarian fossa is not highly vascularized, it does not constitute an optimal location for transplantation.

Recently, the Belgian research team of Jacques Donnez has achieved the first pregnancy in a young female who permitted cryopreservation of ovarian tissue and autotransplantation after thawing. The ovarian fragments, cryopreserved before the patient was exposed to chemotherapy for stage IV Hodgkin's lymphoma, were autotransplanted on the stump of residual ovaries conserved from the time of the intervention. After 5 months from the transplant, the attachment of the transplanted fragments allowed the recovery of ovarian function and 11 months after the transplant the patient spontaneously conceived (44). Although the documentation regarding the fecundated ovum revealed that it had originated from the portion of the ovary which was autotransplanted, there exist some perplexities on the fact that in theory there should have been an ovulation also from the ovarian fragment left in place of the time of the initial procedure. The fact is that what Donnez describes is very similar to the first pregnancy case occurring spontaneously after autotransplantation in its orthotopic position after cryopreservation of ovarian fragments.

Meirow et al. have moreover described, in 2005, the first pregnancy reaching full term after *in vitro* fertilization in a patient of 28 years old affected by a non-Hodgkin's lymphoma, and she had consented to cryopreservation of her ovarian tissue before having been administered high doses of chemotherapy (45). The patient, who had amenorrhea for 24 months after these chemotherapy treatments, underwent an orthotopic reimplantation on the residual ovary and 8 months later she had resumed ovarian function and cyclical menstruation. After ovarian stimulation, withdrawal of oocytes and subsequent *in vitro* fertilization, the transfer in utero of the embryo gave rise to pregnancy resulting in a birth of a healthy baby. In the case described, the possibility for the oocyte obtained to be withdrawn from the residual ovary is very low, given the very high doses of chemotherapy the patient was exposed to and the history of amenorrhea with high levels of gonadotropin at the time of reimplantation.

Experiments involving a heterotopic transplant, in a site different from the natural one (for example in the arm deep to the brachioradialis muscle) (37, 38), have shown encouraging results, with recovery of endocrine function of the ovarian tissue and the menstrual cycle. However, experiments involving *in vitro* fertilization of the herein obtained oocytes have never given rise to pregnancy. However, this procedure is somewhat complicated in nature. Other surgical techniques which can be considered are laparoscopic lateral ovarian transposition or transposing the ovaries behind the uterus and shielding them with a lead block. The latter technique is not only less effective, but it also carries additional risks because the lead block may protect the affected nodes from treatment. Laparoscopic lateral ovarian transposition involves transecting the ovarian ligament and transposing the ovary whilst leaving the fallopian tubes intact. There are reported cases in which the menstrual cycle was never interrupted. In these cases, the ovaries were transposed above the pelvic brim and sutured to the peritoneum (47). Even if this approach is taken, we still recommend that a biopsy of the ovary is taken and cryopreserved to have a "double possibility" just in case.

In case of autotransplants, both orthotopic and heterotopic, it is important to consider the risk of autotransplanting neoplastic cells extracted during the ovarian biopsy; notwithstanding the increased sensitivity of molecular biology techniques available to identify any tumor cells contained within (e.g. Polymerase Chain Reaction or hybridization *in situ*), in the case of some neoplasms (e.g. leukemias) the presence of tumor cells in the ovarian fragments cannot be excluded (35).

In order to minimize the risk of autotransplanting neoplastic cells, alternative strategies for cryopreserved ovarian biopsies have been studied. The following techniques are highly experimental and some, at this stage, remain purely theoretical:

Xenotransplantation is the transplantation of cryopreserved human ovarian fragments in animals of other species lacking immunologic rejection (e.g. SCID mice) (28–30). Naturally, it is necessary even in this case to resort to *in vitro* fertilization of the obtained oocytes.

***In vitro* follicular growth and ovulation (IVM)**. The ovarian fragments or the single primordial follicles obtained through mechanical dissection of the frozen ovarian fragments can theoretically be cultivated *in vitro* until the attainment of mature, fecundable oocytes (39, 40, 41).

Nuclear transfer techniques. The state of the art in this field consists of nuclear transfer techniques, that is the transfer of an immature oocyte nucleus contained within a frozen primordial

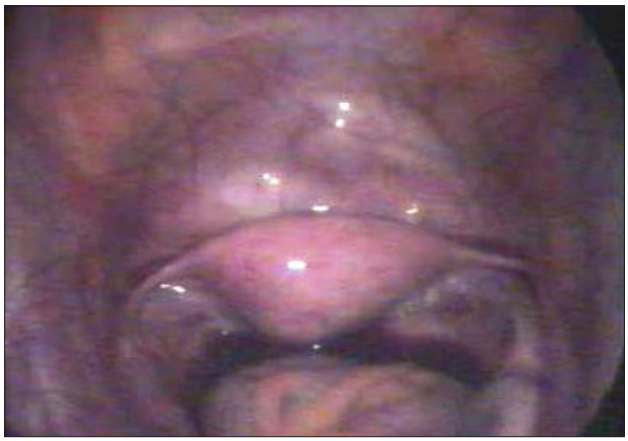

Fig. 1. Endoscopic picture of pelvis before surgery.

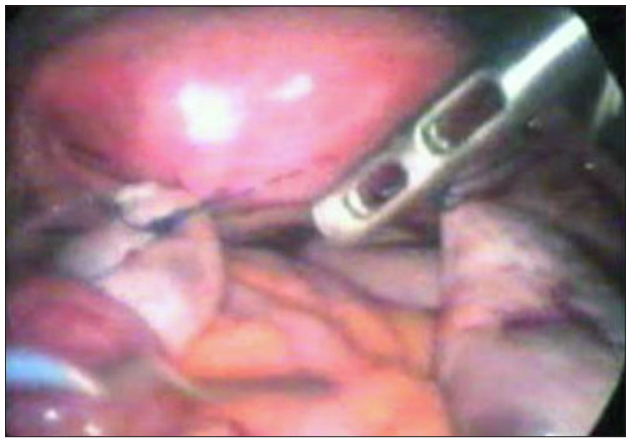

Fig. 2. Left ovary sutured to the uterus.

follicle to the cytoplasm of a fresh oocyte of a donor (42, 43). This futuristic technique offers the possibility to bypass *in vitro* maturation.

Case report

We report a case of a 17-year-old girl who had to undergo pelvic irradiation for non-Hodgkin's lymphoma. To protect the ovaries and preserve fertility, we performed a laparoscopic procedure. We transposed the ovaries behind the uterus (Fig. 1). Using the ovarian ligament as a guy rope, we pierced the ovarian tissue using a needle and passed it through the uterine tissue (Fig. 2). Finally, we sutured the two together. This procedure was performed for both ovaries. Three weeks after the operation, the patient underwent pelvic radiotherapy. The follow-up lasted five months. We performed numerous endocrinological exams: the results were normal and the menstrual cycle was regular. Until now the patient has not conceived spontaneously but it is justified by her age. Nonetheless, we have scheduled regular follow-ups with the patient to see if she will eventually be able to conceive and maintain pregnancy.

Conclusions

Preservation of fertility in girls and young women, especially those that have to undergo oncostatic treatments, is an exigency for patients within an improved health system that takes into account also the quality of their future life.

In most cases antitumoral therapies do not impede reproduction, but in these women diminished fertility, as well as a notable tendency for premature menopause, is frequently observed. Moreover, there are unresolved preoccupations regarding the long-term health of babies conceived from women whose oocytes have undergone antitumoral chemo/radiotherapy. There is a need for a more widespread examination of women and their cryopreserved ovaries after radiotherapy, in order to establish the extent of the genetic damage the oocytes suffered from during the course of such therapies.

The current techniques allow cryopreservation of human ovarian fragments for a long time with good follicular survival rate after thawing. Numerous studies currently being carried out in this field will ultimately improve the survival rate of the oocytes and cryopreserved follicles.

Problems which remain unresolved at present are the choice of the best strategy to embed ovarian fragments after thawing and the way of achieving pregnancy from the same fragments, without risking the reintroduction of the tumoural.

Pregnancies after transplantation of cryopreserved tissue, which have been recently obtained, albeit isolated cases, demonstrate the practicality of preserving female fertility using these methods and instill hope in hundreds of thousands of patients. Currently, however, no conclusive data exists on the efficacy and the absolute guarantee of the procedure, which can vary from case to case in an unpredictable manner.

The following phrase can recapitulate the state of affairs in this field of research: "Now it is time to freeze, time to thaw is coming".

References

1. Deanesly R. Egg survival in immature rat ovaries grafted after freezing and thawing. Proc Roy Soc Lond 1957; 147: 412–421.

2. Parkes AS. Viability of ovarian tissue after freezing. Proc Roy Soc Lond Ser B 1957; 147: 520–528.

3. Parrot DMV. The fertility of mice with orthotopic ovarian grafth derived from frozen tissue. J Reprod Fertil 1960; 1: 230–241.

4. Rutheford AJ, Gosden RG. Ovarian tissue cryopreservation: a pratical option? Acta Paediatr 1999; 433 (Suupl): 13–18.

5. Ataya K, Moghissi K. Chemotherapy induced premature ovatian failure: medications and prevention. Steroids 1989; 54: 607–626

6. Bines J, Oleske DM, Cobleigh MA. Ovarian funcion in premenopausal women treated with adiuvant chemotherapy for breast cancer. J Clin Oncol 1996; 14: 1718–1729.

7. Gradishar WJ, Schilsky RL. Ovarian function following radiation and chemotherapy for cancer. Semin Oncol 1989;16: 425–436.

8. Byrne J, Feurs TR, Gail MH, Pee D, Connelly RR et al. Early menopause in long term survivors of cancer during adolescence. Am J Obstet Gynecol 1992; 166 (3): 788–793.

9. Averette HE, Boyce GM, Girl MA. Effect of cancer chemotherapy on gonadal function and reproductive capacity. Cancer J Clin 1990; 40: 199–209.

10. Blumenfeld Z, Haim N. Prevention of gonadal damage during cytotoxic therapy. Ann Med 1997; 29: 199–206.

11. Ehling UH, Neuhauser-Klaus A. Induction of specific-locus mutation in female mice by 1-ethyl-1-nitrosurea and procarbazine Mut Res 1988; 202: 139.

12. Spinelli S, Chiodi S, Bacigalupo A et al. Ovarian recovery after total body irradiation andallogenic bone marrow transplantation: long term follow-up of 79 female. Bone Marrow Transplant 1994; 14: 373.

13. Lushbaugh CC, Casarett GW. The effects of gonadal irradiation in clinical radiation therapy. A review 1976; 37: 1111.

14. Sanders JE, Hawley J, Levy W et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total body irradiation and bone marrow transplant. Blood 1996; 87 (7): 3045–3052.

15. Wallace WHB, Thomson AB. Preservation of fertility in children treated for cancer. Arch Dis Child 2003; 88: 493–496.

16. Gosden RG. Restitution of fertility in sterilized mice by trasferring primordial ovarian follicles. Hum Reprod 1990; 5: 499–504.

17. Carrol J, Whittingham DG, Wood MJ, Telfert E, Gosden RG. Extra-ovarian production of mature oocytes from frozen primary follicles. J Reprod Fertil 1990; 90: 321–327.

18. Felicio LS, Nelson JS, Gosden RG, Finch CE. restoration of ovulatory cycles by young ovarian grafts in aging mice: potentiation by long-term ovariectomy decreases with age. proc Natl Acad Sci USA 1983; 80: 6076–6080.

19. Nugent D, Newton H, Gallivan L, Gosden RG. Protective effect of vitamin E on ischaemia-reperfusioninjury in ovarian grafts. J Reprod Fertil 1998; 114: 341–6.

20. Bedaiwy MA, Jeremias E, Gurunluoglu R, Hussein MR, Siemianow M, Biscotti C, Falcone T. Restoration of ovarian function after autotransplantation of intact frozen-thawed sheep ovaries with microvascular anastomosis. Fertil Steril 2003; 79 (3): 594–602.

21. Aubard J, Newton H, Piver P, Clavere P, Fermeau V, Gosden RG. The preseration of the follicular population and fertility in irradiated rats by the cryopreservation and orthotopic autografting of ovarian tissue. In: 10th world congress of in vitro fertiliztion and assisted reproduction, Vancouver: Monduzzi Editore, 1997: pp. 99–103.

22. Gosden RG, Baird DT, Wade JC, Webb R. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196°C. Hum Reprod 1994; 9: 597–603.

23. Salle B, Demirci B, Frank M, Rudigoz RC, Guerin JF, Lornage J. Normal pregnancies and live births after autograft of frozen-thawed hemi-ovaries into ewes. Fertil Steril 2002; 77: 403–408.

24. Hovatta O, Silye R, Krausz T et al. Cryopreservation of human ovarian tissue using dimethylsulphoxide and propanediol-sucrose as cryoprotectans. Hum Reprod 1996; 11: 1268–1272.

25. Newton H, Aubard Y, Rutherford A, Sharma V, Gosden RG. Low temperature storage and graftig of human ovarian tissue. Hum Reprod 1996; 11 (7): 1487–1491.

26. Boiso I, Veiga A, Tresserra F et al. Ovarian tissue freezing, a promising method for fertility preservation. Ref Gynecol Obstet 2001; 8: 448–452.

27. Oktay K, Newton H, Gosden RG. Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice. Fertil Steril 2000; 73 (3): 599–603.

28. Gook DA, McCully BA, Edgar DH and McBain JC. Development of antral follicles in human cryopreserved ovarian tissue following xenografting. Hum Reprod 2001; 16: 417–422.

29. Gook DA, Edgar DH, Borg J et al. Oocyte maturation, follicle rupture and luteinization in human ovarian tissue following xenografting. Hum Reprod 2003; 18: 1772–1781.

30. Meirow D, Fasouliotis SJ, Nugent D, Schenker JG, Gosden RG, Rutherford AJ. A laparoscopic technique for obtaining ovarian cortical biopsy specimens for fertility conservation in patients with cancer. Fertil Steril 1999; 71 (5): 948–951.

31. Zhang J, Liu J, Xu KP, Liu B, Di Mattina M. extracorporeal development and ultrarapid freezing of human fetal ova. J Assist Reprod Genet 1995; 12: 361–368.

32. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue N Engl J Med. 2000; 22;342(25):1919.

33. Oktay K, Aydin B, Karlikaya G. A techinque for laparoscopic transplantation of frozen-banked ovarian tissue. Fertil Steril 2001; 75: 1212–1216.

34. Kurokawa T, Kinoshita T, Ito T, Saito H, Hotta T. Detection of minimal residual disease in B cell lymphoma by a PCR-mediated RNase protection assay. Leukemia 1996; 10: 1222–1231.

35. Almodin CG, Minguetti-Camara VC, Meister H, Pigatto Ceschin A, Kriger E and Harri Rodrigues Ferreira JO. Recovery of natural fertility after grafting of cryopreserved germinative tissue in ewes subjected to radiotherapy. Fertil Steril 2004; 81 (1): 160–164.

36. Von Eye Corleta H, Corleta O, Capp E, Edelweiss MI. Subcutaneous autologous ovarian transplantation in Wistar rats mantains hormone secretion. Fertil Steril 1998; 70: 16–21.

37. Oktay K, Economos K, Kan M et al. Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm. J Assoc Am Med Assoc 2001; 286: 1490–1493.

38. Epigg JJ, O'Brien MJ. Development in vitro of mouse oocytes from primordial follicles. Biol reprod 1996; 54: 197–207.

39. Mermillod P, Tomanek M, Marchal R, Meijer L. High developmental competence of cattle oocytes manteined at the germinal vescilce stage for 24 h in colture by specific inhibition of MPF kinase activity. Mol Reprod Develop 2000; 55: 89–95.

40. Zheng P, Si W, Wang H et al. Effect of age and breeding season on the developmental capacity of oocytes from unstimulated and follicle-stimulating hormone-stimulated rhesus monkeys. Biol Reprod 2001; 64: 1417–1421.

41. Liu H, Wang CW, Grifo JA, Krey LC, Zhang J. Reconstruction of mouse oocytes by germinal vescicle transfer: maturity of host oocyte cytoplasm determines meiosis. Hum Reprod. 1999;14 (9): 2357–2361.

42. Moffa F, Comoglio F, Krey LC, Zhang Revelli A, Massobrio M. Germinal vescicle transfer between fresh and cryopreserved immature mouse oocytes. Hum Reprod 2002; 17: 178–183.

43. Bath LE, Wallace WH, Critchley HO. Late effects of the treatment of childhood cancer on the female reproductive system and the potential for fertility preservation. BJOG 2002;109 (2): 107–114.

44. Donnez J, Domans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B, van Langendonckt A et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004; 364 (9443): 1405–1410.

45. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zael Y et al. Pregnancy after Transplantation of Cryopreserved Ovarian Tissue in a Patient with Ovarian Failure after Chemotherapy. N Engl J Med 2005; 6.

46. Anderson B. LaPolla J, Turner D. Chapman G, Buller R. Ovarian transposition in cervical cancer. Gynecol Oncol 1993; 49: 206–214.

47. Bisharah M, Tulandi T. Laparoscopic preservation of ovarian function: An underused procedure. Am J Obstet Gynecol 2003; 188: 367–370.

Received August 25, 2009.
Accepted March 31, 2010.