At what age can human oocytes be obtained?

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Objective: To determine whether oocyte retrieval and in vitro maturation (IVM) is effective in girls undergoing fertility preservation before cancer treatment.
Design: Cohort study.
Setting: Tertiary university medical center.
Patient(s): Patients ≤20 years old before gonadotoxic chemotherapy undergoing ovarian cortex cryopreservation.
Intervention(s): Before ovarian cortex cryopreservation, oocytes in all observed follicles were aspirated, matured in vitro, and cryopreserved.
Main Outcome Measure(s): Maturation of oocytes.
Result(s): One hundred seventy-nine oocytes were detected in 17/19 patients (89%) aged 5–20 years. We found 7, 8, and 17 oocytes in patients 5, 8, and 10 years old, respectively. The median number of oocytes per patient was 9 (0–37). Maturation rate was 45/133 oocytes (34%). In total, 81 oocytes were cryopreserved. We cryopreserved 4 of 12 detected, 4 of 9 detected, 1 of 8 detected, and 4 of 9 detected IVM oocytes for patients aged 5–10, 11–14, 15–17, and 18–20 years old, respectively.
Conclusion(s): Patients undergoing ovarian cryopreservation could benefit from supplementary oocyte aspiration from the cortex. Surprisingly, oocytes were detected even in young premenarcheal girls. The number of oocytes detected, matured, and cryopreserved was not age dependent. Retrieved oocytes can be matured in vitro and cryopreserved. Because no pregnancy has yet resulted from this procedure it should be considered to be experimental. We describe the youngest patients to undergo ovum collection, IVM, and oocyte cryopreservation. (Fertil Steril® 2009;92:458–63. ©2009 by American Society for Reproductive Medicine.)

Key Words: Fertility, premature ovarian failure, pediatric hemato-oncology, cryopreservation, ovary, chemotherapy, in vitro maturation, laparoscopy

Advances in early detection and increasing success of chemotherapy have made cancer a curable disease. In children and adolescents with cancer, cure rates approach 75%. These cure rates are achieved in great part as a result of the use of intensive chemotherapy, and in some cases, radiation. The use of these treatment modalities is associated with significant toxicity, including the potential for gonadal damage and subsequent reduced fertility. Aggressive chemotherapy is, however, usually gonadotoxic and results in infertility in many pediatric patients. Ovarian damage is drug and dose dependent (1) and increases with patient's age at treatment (2). Increasing numbers of young cancer survivors are therefore experiencing infertility related to their past cancer treatment. Having children thus becomes an important issue for young cancer patients (3).

Cryopreservation of sperm is an effective method that is offered to pre- and postadolescent males (4). Mature female gametes were, however, not readily amenable to cryopreservation, although the use of vitrification recently resulted in improved results (5). Nevertheless, this method is not applicable for young girls as it requires prolonged induction of ovulation and vaginal sonography to complete aspiration of oocytes. Similarly, IVF may be offered only to patients beyond adolescence. Ovulation induction requires a few weeks' delay in the initiation of cancer treatment.

Because ovarian stimulation is generally not a feasible option for young girls and adolescents, strategies for preserving fertility in these patients usually include ovarian cryopreservation, an experimental technology with some success in

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animal studies, recently resulting in few deliveries after human transplantations (6, 7). Although the technique remains investigational, it is being increasingly offered to women undergoing cancer treatment. In prepubertal girls ovarian cryopreservation is the only option for potentially preserving ovarian function (8). As we and other investigators have shown, it is probable that methods of ovarian transplantation with vascular anastomosis will be applied in the future (9–12).

We have recently recommended that following individual consultation by a multidisciplinary team, all female pediatric cancer patients and their families should be counseled regarding side effects of chemotherapy and be offered ovarian preservation (13).

The methodology of ovarian cortex preservation, pioneered by Gosden et al. (14) is currently routine in many centers in a few countries (15). Nevertheless, it is realized that the future use of this cryopreserved ovarian cortex may be limited because of cellular injury during cryopreservation and the tissue ischemic damage after transplantation (16).

Furthermore, cryobanking of ovarian cortex preserves only the smallest (primordial and primary) follicles, as all preovulatory antral follicles, which contain germinal vesicle (GV) stage oocytes will not survive the procedure (17). We thus currently propose to all patients undergoing ovarian cryopreservation to perform integrated oocyte aspiration from antral follicles of the tissue, followed by in vitro maturation (IVM) and oocyte cryopreservation as an additional fertility-preserving method (18). The aim of this study was to analyze oocyte detection and IVM success rates in young girls and adolescents using this combined method.

MATERIALS AND METHODS

After referral from the Hadassah Pediatric Hematology-Oncology department the patients were consulted with their parents. This included an evaluation of their general status, an abdominal ultrasound to evaluate the ovaries, and an explanation on the effect of chemotherapy on future fertility potential. Methods to preserve female fertility were presented, as well as the pros and cons of each. A discussion was then carried out with the pediatric hematologist-oncologist and the bone marrow transplantation team to consider the best recommendation for each patient (13). The study was approved by the Hadassah hospital ethics committee. Serological testing for hepatitis (B and C) and HIV was done. Surgery was scheduled in combination with other procedures such as bone marrow biopsy or insertion of totally implantable venous access system.

Surgery

Laparoscopic unilateral oophorectomy was performed using a harmonic scalpel (Johnson & Johnson, Kibbutz Shefayim, Israel). The ovary was removed from the abdomen in a single use specimen retrieval pouch and immediately transferred on ice to the IVF laboratory in Leibovitz medium (GIBCO-BRL, Paisley, United Kingdom) and placed under a dissecting microscope.

FIGURE 1

Aspiration of oocytes.

Revel. Oocyte IVM and cryopreservation in girls. Fertil Steril 2009.

Retrieval of Immature Oocytes From Ovaries

Antral follicles observed on the ovarian surface were aspirated (Fig. 1) before tissue cryopreservation by using a 1-mL syringe and a 19-gauge needle and flushed with HEPES-buffered human tubal fluid (HTF) medium (Irvine Scientific, Santa Ana, CA) containing 10% synthetic serum supplement (SSS; Irvine Scientific). All antral follicles observed on the ovarian surface were aspirated as previously detailed (18). Cumulus–oocyte complexes and the naked oocytes that emerged from the follicles were collected with micropipettes and incubated in P1 medium (Irvine Scientific) supplemented with 10% SSS. After the dissection of the ovarian tissue, the remaining media dishes were searched for cumulus–oocyte complexes as well.

In Vitro Maturation

All cumulus–oocyte complexes recognized were transferred into 1-mL culture dishes containing 10% P1 medium, and incubated at 37°C in an atmosphere of 90% N_2, 5% CO_2, and 5% O_2.

During the initial phase of the study, oocytes were denuded of the surrounding cumulus cells using hyaluronidase solution within the first 2–4 hours of incubation and assessed for maturity. The denuded oocytes were matured by incubation in either 10% P1 or homemade IVM culture media.

More recently, cumulus–oocyte complexes were left intact. Complexes were incubated in 10% P1, homemade IVM or SAGE IVM culture media (SAGE, Trumball, CT). Denudation of oocytes was performed 24 hours later. Oocytes that did not mature were incubated for an additional 24 hours.

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Cryopreservation of Oocytes

Oocyte cryopreservation was carried out by slow freezing according to the method described by Chen et al. (19), as was previously described. In brief, freezing and thawing solutions were prepared by using Dulbecco's phosphate-buffered solution (PBS; Irvine Scientific), 1,2-propanediol (PROH) (Sigma, St. Louis, MO), and 12 mg/mL human serum albumin (has). The freezing solutions prepared were 1.5 M PROH + 12 mg/mL HSA in PBS (equilibration solution) and 1.5 M PROH + 12 mg/mL HSA + 0.3 M sucrose (Sigma) in PBS (freezing solution).

Oocytes were first washed in a 100-μL droplet of PBS supplemented with 12 mg/mL HSA. Up to five oocytes were then placed in a 100-μL droplet of equilibration solution for 10 minutes in room temperature, followed by transfer to a 100-μL droplet of the freezing solution for an additional 15 minutes. Oocytes were then loaded into cryovials containing 250 μL of the freezing solution and placed into an automated Kryo10 series biological freezer (Planer Kryo; Planer PLC, Middlesex, United Kingdom). The initial chamber temperature was 23°C. The temperature was reduced to −7°C at a rate of 2°C/min. After a 10-minute soaking, manual seeding was induced at −7°C, followed by a 15-minute holding time. The chamber was further slowly cooled to the temperature of −30°C at a rate of 0.3°C/min, followed by rapid cooling to −140°C at a rate of 10°C/min. The ampules were transferred into liquid nitrogen containers for storage.

Cryopreservation of Ovarian Cortex

The ovarian cortex was prepared and cryopreserved according to appropriate protocols (20). Briefly, the ovary is cut in two along its long axis. The cortex is thinned until transparent by removing the medulla. This procedure should be performed quite rapidly in cold (4°C) Leibovitch L-15 medium on ice. Using a scalpel, slivers of 5 mm^2 (5 x 1 x 1) were prepared and transferred to 2-mL tubes for 30-minute equilibration at 4°C with cryoprotectant consisting of 1 mL of 1.5 M dimethyl sulfoxide (DMSO), 0.1 M sucrose, and 10% (vol/vol) HSA in (4°C) Leibovitch L-15 medium. Ovarian slices were transferred to cryotubes containing 1 mL of the cryoprotectant, and transferred into an automated Kryo10 series biological freezer (Planer Kryo; Planer PLC). 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. Cryotubes were then transferred into liquid nitrogen containers.

RESULTS

During the study period 19 patients aged 5–20 years old underwent laparoscopic oophorectomy for ovarian cryopreservation. Patient's age, diagnoses, and surgical procedures performed in parallel to oophorectomy are detailed in Table 1. The median patient's age in the study group was 15 years (range 5–20 years). All patients were operated by laparoscopy with no adverse effects during surgery or postoperatively and were discharged within 24 hours of surgery. The delay in onset of chemotherapy was no more than 2 days.

One ovary was successfully removed from all patients. The time to aspirate all observed follicles and search for cumulus–oocyte complexes was 10–15 minutes.

One hundred seventy-nine oocytes were detected in 17/19 (89%) patients. The median number of oocytes retrieved per patient was 9 (0–37). In two patients (aged 16 and 20 years) no oocytes were detected and we cryopreserved only their ovarian cortex (6 and 15 ampules, respectively). For example, we found 7, 8, and 17 oocytes in 5 (Wilm's tumor), 8 (Ewing sarcoma) (Fig. 2), and 10-year-old (Ewing sarcoma) patients, respectively.

In one (19 years old) patient we retrieved 37 oocytes; however, she refused to submit them to IVM and requested to cryopreserve all her oocytes, 2 of which were found to be mature. In one other patient one oocyte was found to be mature on the day of collection, thus collectively three oocytes were mature (1.7%). Also not submitted to IVM were eighth oocytes (4.5%) that were degenerative. The remaining 133 GV stage oocytes were submitted to IVM.

Oocyte In Vitro Maturation and Cryopreservation

The overall maturation rate by IVM was 34% (45/133 oocytes). In four patients no maturation was achieved. Although some oocytes matured within a day, most maturation was seen on day 2. In total, 81 oocytes were cryopreserved, of which 45 were mature, 4 were germinal vesicle breakdown (GVBD), and 32 were at the GV stage.

The maximal number of oocytes submitted to IVM in the study was 18 and 17 in a 15- and a 10-year-old patient, respectively. In girls 5–10 years old we cryopreserved a mean of 4 from 12 detected oocytes (33.3%). Similarly, we in vitro matured and cryopreserved on average 4 of 9 detected, 1 of 8 detected, and 4 of 9 detected, for patients aged 11–14, 15–17, and 18–20 years, respectively. Thus it appears that a similar number of oocytes could be retrieved and matured in young premenarcheal girls.

Ovarian Cortex Cryopreservation

In addition to the cryopreserved oocytes all patients had preservation of an average of 12 (6–17) ampules containing slivers of ovarian cortex (about 6 pieces per ampule).

Patient Follow-Up

Four patients passed away either from disease (N = 2) or complications of chemotherapy (N = 2; aplastic anemia, graft versus host disease). Normal menarche occurred in 1 of the 4 premenstrual patients and 4 of 11 (36%) postmenarchal patients became amenorrheic after therapy.

DISCUSSION

The youngest mother in the world reported is Lina Medina, a Peruvian girl who gave birth by cesarean section on May

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TABLE 1

Clinical characteristics and follow-up of patients.

Age at treatment (y)	Diagnosis	Additional operative procedure	Number of retrieved oocytes	Number of frozen oocytes	Ovarian ampules frozen	Follow-up	Menstruation before/after
5	Wilms tumor		7	1	9	Alive, on therapy	No/no
8	Ewing sarcoma	TIVAS	8	2	8	Alive after relapse	No/no
10	Osteosarcoma	TIVAS	17	8	11	Alive, on therapy	No/no
13	Hodgkin's disease		2	2	10	Alive and well	No/yes
13	Ewing sarcoma	BM biopsy	9	5	12	Died of AA	
14	Ewing sarcoma	BM biopsy	16	6	12	Alive after relapse	Yes/no
15	AML		5	—	9	Alive and well	Yes/no
15	Osteosarcoma		18	2	12	Alive and well	Yes/yes
16	Thalassemia		4	2	12	Alive and well	Yes/no
16	Hodgkin's disease		9	4	11	Alive and well	Yes/yes
16	Ovarian GC tumor		0	—	6	Alive and well	Yes/yes
16	Ewing sarcoma	BM biopsy	1	1	12	Died of disease	
17	NHL		10	—	14	Alive, on therapy	Yes/yes
18	Hodgkin's disease		10	—	12	Alive, on therapy	Yes/yes
19	Ewing sarcoma	T&BMb	37	37	12	Alive and well	Yes/no
20	Hodgkin's disease		9	3	14	Alive, on therapy	Yes/yes
20	Thalassemia		5	3	16	Died of GVHD	
20	NHL		0	—	15	Died of disease	
20	Hodgkin's disease		12	5	17	Alive, on therapy	Yes/yes

Note: AA = aplastic anemia; AML= acute myelocytic leukemia; BM = bone marrow, GC = germ cell; GVHD = graft versus host disease; NHL= non Hodgkin's lymphoma; TIVAS= totally implantable venous access system; T&BMb = TIVAS and BM biopsy.

Revel. Oocyte IVM and cryopreservation in girls. Fertil Steril 2009.

14, 1939, at the age of 5 years (21). Her case was reported in detail by Dr. Edmundo Escomel, including the pathology of ovarian biopsy during her cesarean section and reports adult-looking ovarian tissue (22). Although she may have suffered from precocious puberty, this supports the possibility of oocytes to mature and ovulate at age 5 years.

To the best of our knowledge we report here the youngest age for ovarian oocyte retrieval. We were surprised to find oocytes in 5- and 8-year-old girls (7 and 8 oocytes, respectively). In both of these girls as well as in older ones we managed to cryopreserve mature oocytes after IVM. In addition, the number of oocytes obtained and the maturation rate does not seem to be affected by age.

In theory, GV immature oocytes, lacking a mitotic spindle, should be easier to cryopreserve. Good results were obtained when immature sheep oocytes were vitrified (23). In contrast, immature bovine oocytes showed cryoprotectant concentration, type, and exposure time to affect the oocyte competence and development after vitrification (24). Similarly, poor maturation and subsequent embryonic development of frozen-thawed GV stage oocytes were noted to be associated with immature oocyte cryopreservation (25, 26). Only one human

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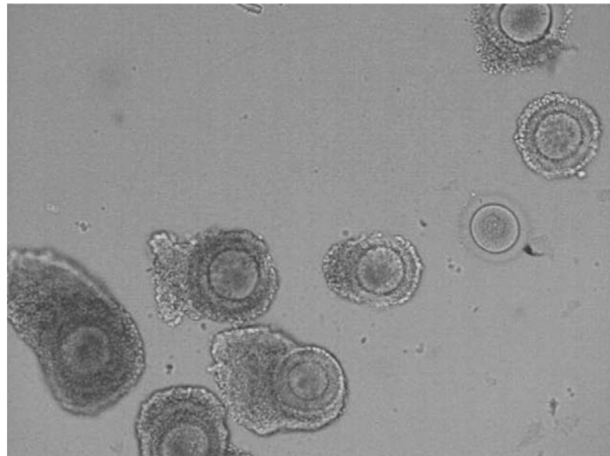
FIGURE 2

Retrieved oocytes.



Revel. Oocyte IVM and cryopreservation in girls. Fertil Steril 2009.

pregnancy was reported in a tubal infertility patient undergoing IVF in which immature oocytes were cryopreserved and later matured in vitro (27).

Our preliminary data suggested that oocytes should be matured before cryopreservation, and thus we have applied this approach. A recent report from McGill (28) described oocyte aspiration, IVM, and vitrification in four patients (18–38 years old). The number of retrieved oocytes was somewhat lower (average 2.75); however, the high maturation rate has resulted in vitrification of one to three oocytes per patient. The recent literature supports the shift toward vitrification of human oocytes (29, 30). It should be stressed that the efficacy of this approach is low as only oocytes are detected, matured, and cryopreserved. We thus offer this only in the parallel to ovarian cryopreservation in young women. This process may be even less efficacious in girls with smaller numbers of oocytes.

The decision to propose ovarian cryopreservation in young female patients should be based on the presumed risk of ovarian failure after cancer treatment, the general condition of the patient, as well as her and her guardian's preference. These considerations should be assessed by a multidisciplinary team consisting of pediatric hematologist-oncologists, the fertility expert, surgeons, and anesthesiologists (13). We believe that the patients; best interest is served as this appears to give them not only a possibility to restore fertility but also conveys a message of hope for a better future, looking into the long-term possibility of parenthood. The Edinburgh, United Kingdom, criteria for selection of patients for whom this approach is most suitable include girls at high risk of treatment-induced immediate ovarian failure, realistic chance of long-term survival, and consent by the patient and her parents (31).

We consider that the surgical and IVM techniques developed following our preliminary experience (18) are satisfactory. Recent reports support our approach of combining ovarian tissue cryobanking with retrieval of immature oocytes followed by IVM and vitrification as an additional strategy of fertility preservation for patients with cancer (28) or Turner's syndrome (32). Nevertheless, no data are available concerning the viability of the matured oocytes after cryopreservation for adult women or for girls. Although survival and pregnancy rates (PR) after oocytes cryopreservation are low, they do seem to be on the rise (5).

The use of a harmonic scalpel significantly shortened the time to obtain the ovary, does not cause thermal damage to the ovarian cortex, and shortened the laparoscopic procedures. Feigin et al. recently reported that a harmonic scalpel is safe and useful for this procedure in young patients (33).

We removed one whole ovary as we considered the infertility risk to be greater when ovaries were exposed to intensive chemotherapy than that of unilateral oophorectomy. Indeed fertility of patients with one ovary appears not to be reduced (34). In addition, it appears that especially for young patients with smaller ovaries, a sufficient amount of ovarian cortex can be obtained only through the removal of one whole ovary as a large number of ovarian follicles will not survive cryopreservation and transplantation (35).

In conclusion, we found that young girls and adolescents diagnosed with cancer should be counseled by a multidisciplinary team including pediatric hematologist-oncologists, anesthesiologists, and fertility specialists. Laparoscopic oophorectomy is well tolerated by these patients and has resulted in the cryopreservation ovarian cortex. Oocytes extracted during ovarian cryopreservation from adult women can be matured and cryopreserved with or without fertilization by sperm, as they will not survive cryopreservation inside the ovarian cortex. Because this is an experimental approach, we consider that this approach should be cautiously presented to girls and adolescents.

We consider that this approach not only gives these girls a realistic chance of pregnancy after gonadotoxic chemotherapy but also a source of hope for the young patient and her parents.

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REFERENCES

1. Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. Cancer 2007;110:2222–9.
2. Damewood MD, Grochow LB. Prospects for fertility after chemotherapy or radiation for neoplastic disease. Fertil Steril 1986;45:443–59.
3. Schover LR. Motivation for parenthood after cancer: a review. J Natl Cancer Inst 2005;2–5.
4. Revel A, Haimov-Kochman R, Porat A, Lewin A, Simon A, Laufer N, et al. In vitro fertilization–intracytoplasmic sperm injection success rates with cryopreserved sperm from patients with malignant disease. Fertil Steril 2005;84:118–22.
5. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil Steril 2006;86:70–80.

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6. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;364:1405–10.
7. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005;353: 318–21.
8. Salha O, Picton H, Balen A, Rutherford A. Cryopreservation of human ovarian tissue. Hosp Med 2001;62:222–7.
9. Arav A, Revel A, Nathan Y, Bor A, Gacitua H, Yavin S, et al. Oocyte recovery, embryo development and ovarian function after cryopreservation and transplantation of whole sheep ovary. Hum Reprod 2005;20:3554–9.
10. Bedaiwy MA, Falcone T. Ovarian tissue banking for cancer patients: reduction of post-transplantation ischaemic injury: intact ovary freezing and transplantation. Hum Reprod 2004;19:1242–4.
11. Martinez-Madrid B, Dolmans MM, Van Langendonckt A, Defrere S, Donnez J. Freeze-thawing intact human ovary with its vascular pedicle with a passive cooling device. Fertil Steril 2004;82:1390–4.
12. Revel A, Elami A, Bor A, Yavin S, Natan Y, Arav A. Whole sheep ovary cryopreservation and transplantation. Fertil Steril 2004;82:1714–5.
13. Weintraub M, Gross E, Kadari A, Ravitsky V, Safran A, Laufer N, et al. Should ovarian cryopreservation be offered to girls with cancer. Pediatric Blood & Cancer 2007;48:4–9.
14. Gosden RG, Baird DT, Wade JC, Webb R. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196 degrees C. Hum Reprod 1994;9:597–603.
15. Donnez J, Bassil S. Indications for cryopreservation of ovarian tissue. Hum Reprod Update 1998;4:248–59.
16. Candy CJ, Wood MJ, Whittingham DG. Effect of cryoprotectants on the survival of follicles in frozen mouse ovaries. J Reprod Fertil 1997;110:11–9.
17. Gosden RG. Gonadal tissue cryopreservation and transplantation. Reprod Biomed Online 2002;1(4 Suppl):64–7.
18. Revel A, Koler M, Simon A, Lewin A, Laufer N, Safran A. Oocyte collection during cryopreservation of the ovarian cortex. Fertil Steril 2003;79:1237–9.
19. Chen SU, Lien YR, Chen HF, Chang LJ, Tsai YY, Yang YS. Observational clinical follow-up of oocyte cryopreservation using a slow-freezing method with 1,2-propanediol plus sucrose followed by ICSI. Hum Reprod 2005;20:1975–80.
20. Gosden RG. Low temperature storage and grafting of human ovarian tissue. Mol Cell Endocrinol 2000;163:125–9.
21. Wikipedia. Lina Medina. Available at: Wikipedia 2007;http://en.wikipedia.org/wiki/Lina_Medina. Accessed 9 March 2008.
22. Escomel E. L'ovaire de Lina Medina, la plus jeune mere du monde. Press Med 1939;47:1648.
23. Bogliolo L, Ariu F, Fois S, Rosati I, Zedda MT, Leoni G, et al. Morphological and biochemical analysis of immature ovine oocytes vitrified with or without cumulus cells. Theriogenology 2007;68:1138–49.
24. Yamada C, Caetano HV, Simoes R, Nicacio AC, Feitosa WB, Assumpcao ME, et al. Immature bovine oocyte cryopreservation: comparison of different associations with ethylene glycol, glycerol and dimethylsulfoxide. Anim Reprod Sci 2007;99:384–8.
25. Toth TL, Lanzendorf SE, Sandow BA, Veeck LL, Hassen WA, Hansen K, et al. Cryopreservation of human prophase I oocytes collected from unstimulated follicles. Fertil Steril 1994;61:1077–82.
26. Son WY, Park SE, Lee KA, Lee WS, Ko JJ, Yoon TK, et al. Effects of 1,2-propanediol and freezing-thawing on the in vitro developmental capacity of human immature oocytes. Fertil Steril 1996;66:995–9.
27. Tucker MJ, Wright G, Morton PC, Massey JB. Birth after cryopreservation of immature oocytes with subsequent in vitro maturation. Fertil Steril 1998;70:578–9.
28. Huang JY, Tulandi T, Holzer H, Tan SL, Chian RC. Combining ovarian tissue cryobanking with retrieval of immature oocytes followed by in vitro maturation and vitrification: an additional strategy of fertility preservation. Fertil Steril 2008;89:567–72.
29. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology 2007;67:73–80.
30. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod Biomed Online 2006;12: 779–96.
31. Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol 2005;6:209–18.
32. Huang JY, Tulandi T, Holzer H, Lau NM, Macdonald S, Tan SL, et al. Cryopreservation of ovarian tissue and in vitro matured oocytes in a female with mosaic Turner syndrome: case report. Hum Reprod 2008;23:336–9.
33. Feigin E, Abir R, Fisch B, Kravarusic D, Steinberg R, Nitke S, et al. Laparoscopic ovarian tissue preservation in young patients at risk for ovarian failure as a result of chemotherapy/irradiation for primary malignancy. J Pediat Surg 2007;42:862–4.
34. Lass A. The fertility potential of women with a single ovary. Hum Reprod Update 1999;5:546–50.
35. Abir R, Fisch B, Nahum R, Orvieto R, Nitke S, Ben Rafae Z. Turner's syndrome and fertility: current status and possible putative prospects. Hum Reprod Update 2001;7:603–10.

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