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Residual Fertility in Childhood Cancer Survivors

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Abstract: During the past three decades, major improvements have been made in the treatment and cure of certain hematological malignancies, as well as solid tumors in young patients. As a result of improved survival, attention has been turned to the long-term physical and psychological sequelae of treatment. The loss of fertility in males and premature menopause in females are important and common long-term side effects of curative radio- and chemotherapy. The frequency of fertility failure varies with the type, dose, duration of radio- and chemotherapy, and age of patient. Currently, there are no good estimates of the magnitude of the risk involved in relation to these factors. However, the combination of hormonal values, pubertal staging and the ultrasonographic and Doppler analyses of the gonads may noninvasively study the subtle modification following anticancer therapies. This could help to find new insights on potential preventive acts before initiation of the anticancer therapy, and hopefully, the restoration of fertility after treatment.

Key words: Radiotherapy, chemotherapy, gonadal function, spermatogenesis, spermatid, testicular function, uterine function cryopreservation, radiation doses.

INTRODUCTION

Pediatric tumors differ greatly from adult cancers in terms of their origin, distribution and prognosis. Only 1% of all new cases in the United States affect children. However, malignancies are the principal cause of death among children aged 1 to 15 years.[1] Acute lymphocyte leukemia (ALL), brain tumors and sarcoma are more frequent in children, whereas acute and chronic myeloid leukemia, chronic lymphoid leukemia and carcinomas occur mostly in adults. The distribution of tumors changes during childhood through adolescence: the most common tumors in children of <5 years are ALL (36%), lymphoma (10%), kidney (10%) and brain tumors (13%). Between age 5 and 9 years ALL slightly decreases (31%), lymphoma (16%) and brain tumors increase (25%) and kidney tumors represent only 5%. Between age 10 and 14 years ALL, lymphoma and brain tumors are 18%, 25% and 18%, respectively, of all malignancies. Bone tumors become more frequent (11%).[1]

In the past, childhood malignancies were rapidly and inevitably fatal. However, of the approximately 7,500 children under 15 years of age who are found to have cancer in the United States each year, some 80% (or nearly 6,000), can expect to be cured of their diseases. It has been estimated that the prevalence of childhood cancer survivors (15 to 45 years of age) in the United States will increase from 1 in 1000 persons in 1990, to 1 in 900 persons in 2000 and, possibly, as many as 1 in 250 persons in the year 2010.[2] This is primarily due to an improvement in the combinations of surgery, radiotherapy (RT), and intensive multi-agent chemotherapy (CT).[3] However, cancer is not a single disease and hidden within these overall figures are substantial differences between cancers in terms of overall survival. For ALL, the most common malignancy, survival is around 73%. For a few cancers, the survival rates are above 90% (Hodgkin's disease, retinoblastoma, germ cell tumors). Unfortunately, less success has been achieved with other cancers (central nervous system tumors or neuroblastoma).[4] With the improvement of survival, it has become more and more obvious the need to screen survivors of childhood cancer for late effects of cancer therapy, because almost half of these subjects are likely to have or to develop disabilities that alter quality of life.

The possibility of severe gonadal damage is one of the more frequent sequelae as both male and female gonads are sensitive, although in different ways, to the damage-induced both by CT and irradiation. While CT has prevalent lesive effect on the testes, irradiation is responsible for severe damage to both testicular and ovarian functions.

CHEMO/RADIOTHERAPY AND GONADAL FUNCTION

Males

Spermatogenesis is a process, beginning at puberty and continuing throughout adult life, whereby totipotential stem cell, i.e. spermatogonia undergo continual self-renewal and differentiation into mature spermatozoa.

It had been suggested that CT-induced damage is proportional to the degree of gonadal activity during treatment and that prepubertal children with inactive gametogenesis should have a lower incidence of injury.[5] However, increasing evidence suggests that although testicular quiescence confers some degree of protective effect, most prepubertal boys starting from midpuberty, show the classical features of gonadal damage.[6,7] Age of treatment, therefore, does not represent an important variable and the degree of gonadal injuries mostly depends on the characteristics of the CT agents, the duration of treatment, and dosage of the drugs.[8] However, as most treatments are delivered as multi-agents regimen with often synergistic toxicity, it can be difficult to determine the specific contribution of each individual agent.

Alkylating agents (procarbazine, mechlorethamine, cyclophosphamide, chlorambucil, busulfan, etc.) have been identified as gonadotoxic.[9] By acting as inhibitors of DNA synthesis they damage, in particular, cells with rapid mitotic activity (germinal cells of the testicular tubules), leading to severe germinal aplasia and adulthood oligo-azoospermia.[10]

Cyclophosphamide and chlorambucil do have dose dependent effects, but are reversible in up to 70% of patients after therapy-free intervals of several years.[6] On the contrary, in pubertal males treated with MOPP (mechloethamine, vincristine, prednisone and procarbazine) or COPP (cyclophosphamide, vincristine, prednisone and procarbazine) evaluated 1 to 2 years after completion of therapy, azoospermia is found in 80 to 100% of the cases.[11] This effect is reversible in only about 20% of cases even 7 years after therapy.[11] The percentage may increase with fewer MOPP cycles and may decrease with pelvic irradiation. With the ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) combination that contains no alkylating agent nor procarbazine, the incidence of azoospermia appears to be lower (36%) and the incidence of recovery higher than after MOPP (100%).[11] Alternate cycles of ABVD with cycles of MOPP, are likely to be less gonadotoxic. Fertility is preserved in approximately 50% of patients following three cycles of MOPP/ABVD, compared to almost universal azoospermia following six cycles of MOPP.[11]

To an even greater extent than its effect on spermatogenesis, the effects of MOPP on Leydig cell function appear to be age-related. The MOPP therapy, prior to the onset of puberty, leads to a normal pubertal progression. Gynecomastia with low testosterone and increased LH is developed in patients treated during adolescence. Compensated Leydig cell failure (increased LH with low normal testosterone levels or exaggerated FSH and LH response to GnRH) without gynecomastia is seen in adults.[12] However, in some patients treated during childhood, as they face puberty, LH values are higher than normal with testosterone levels that are at the lowest limit of the normal range. It has been suggested that this state of slight insufficiency of testosterone release is responsible for reduced bone mineral density in adulthood.[13]

The testis is one of the most radiosensitive tissues with very low doses of radiation causing significant impairment of function. Damage can be caused during direct irradiation of the testis or, more commonly, from scattered radiation during treatment at adjacent tissues. The more immature cells are more radiosensitive with doses as low as 0.1 Gy, causing morphological and quantitative changes to spermatogonia. Doses of 2 to 3 Gy result in overt damage to spermatocytes leading to a reduction in spermatid numbers. At doses of 4 to 6 Gy, numbers of spermatozoa are significantly decreased implying damage to spermatid.[14] Recovery of spermatogenesis takes place from surviving stem cells (type A spermatogonia) and is dependent on the dose of radiation. Complete recovery, as indicated by a return to pre-irradiation sperm concentrations and germinal cell numbers, takes place within 9 to 18 months following 1 Gy or less, 30 months after 2 to 3 Gy, and 5 years or more for doses higher than 4 Gy.[14]

It has been suggested that fractionation of radiotherapy increases gonadal toxicity.[15] Doses of 1.2 Gy may represent a threshold for permanent testicular damage. Testicular doses of less than 0.2 Gy had no significant effect on FSH levels or sperm counts, whilst doses between 0.2 and 0.7 Gy caused a transient dose-dependent increase in FSH and reduction in sperm count, with a return to normal values within 12 to 24 months.[16]

Leydig cells are more resistant to injury by irradiation, but a high dose (>20 Gy) direct to the testes provokes severe testicular damage that will be followed not only by sterility, but also by impaired testosterone secretion.[17] Most of these boys will therefore, require replacement therapy with testosterone to permit normal pubertal development and sexual activity.[17]

Investigation of Testicular Function

The only direct way to assess spermatogenesis is by semen analysis. However, in young males it is not always possible, for various reasons such as ethics, religion or shame, to obtain a semen sample. Therefore, it is very important to find indirect, but reliable ways to assess the effect of CT and RT on gonadal function.

Pubertal staging provides indirect information about Leydig cell function and spermatogenesis.[18] The development of normal secondary sexual characteristics implies intact Leydig cell function with normal steroidogenesis. A reduced testicular volume (<15 ml, using the Prader Orchidometer), suggests impaired spermatogenesis.[18]

Hormone analysis should include the basal value of FSH, LH and testosterone. In prepubertal children hormonal evaluation is not as useful since the hypothalamic-pituitary-

testicular axis is silent. In postpubertal boys, elevated LH and reduced testosterone levels indicate Leydig cell dysfunction. Elevated FSH with normal testosterone levels indicate germ cell failure.[19]

Ultrasonography of the testis provides information on tissue morphology (in a gray scale) and enables to calculate the testicular volume more accurately than with the Prader orchidometer (Fig. 1). The simultaneous addition of color Doppler provides informations on blood flow (in color). Recent reports demonstrated a direct correlation between FSH and testicular vascularization, and an inverse correlation between the testicular volume and the pulsatility index of the transmediastinal artery (TMA).[20,21] Thus, in absence of a semen analysis, the combination of hormonal values, pubertal staging and ultrasound study of the testis may predict the testicular damage provoked by CT/RT.

It has been shown that children treated with higher doses of cyclophosphamide, procarbazine, and dacarbazine, present a more evident testicular damage as confirmed by significantly higher FSH levels, lower testicular volume, and more elevated TMA resistances (Fig. 2).[22] As the seminiferous tubules represent approximately 80 to 85% of the testicular mass, a small testicular volume and elevated FSH may suggest a significant impairment of the seminiferous tubules with subsequent testicular fibrosis, damaged spermatogenesis, and inhibin deficiency.[23] In a recent study,[22] TMA resistances resulted in higher rates in patients treated with higher cytotoxic doses (Fig. 3). Since the testicular mediastinum is the entry site for the main testicular vessels and PI is used as a measure of blood flow impedance distal to the point of sampling, elevated PI values may express a low testicular vascularization. The authors speculated that in patients with childhood malignancies treated with CT/RT, the severely altered testicular structure could be associated with fibrotic processes and significant decrease of blood flow supply to the testes.[22] In fact, these patients showed a high testicular texture score, which is known to be correlated with a low number of germinative tubules and an increased number of obliterated tubules,[24] and with a reduced sperm count.[25]

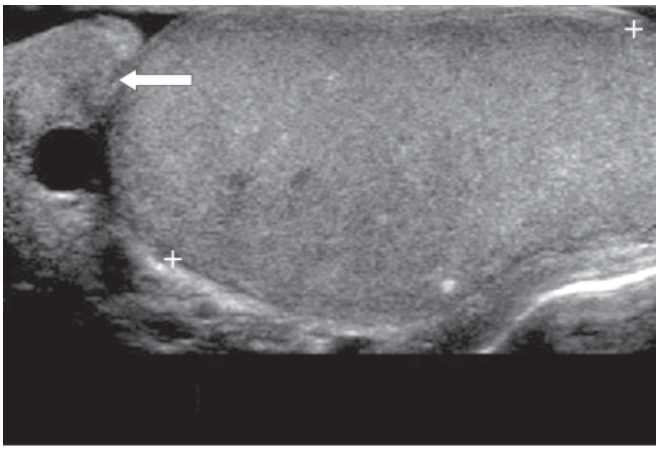

Fig. 1: Ultrasonographic representation of the didymis (comprised between the two crosses) and of the epididymis (arrow).

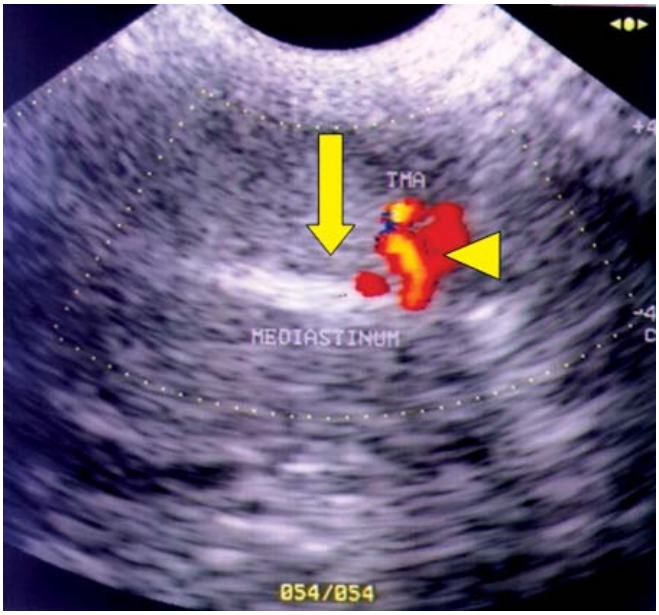

Fig. 2: Color flow image of the transmediastinal artery (TMA). The TMA is sampled in longitudinal plane at the level of the testicular mediastinum. (arrow = testicular mediastinum; triangle = TMA)

Females

Chemotherapy causes both early and late effects which limits the dose and schedule of exposure. The ovary is chemosensitive, but there are no data on threshold doses for ovarian dysfunction.

The number of primordial follicles reaches a peak around six months intrauterine life at approximately 7×10^6 follicles. There is an exponential decline in follicles throughout infancy to adulthood by atresia, with only approximately 400 being released as mature oocytes. When the number of remaining primordial follicles falls below one thousand, the menopause occurs.[26]

There is a relationship between the age of menopause and childhood cancer survivors and the treatment to which they have been exposed. A review of 1,067 women who were more than 5 years on treatment for cancer, diagnosed during the teenage years, demonstrated a risk of menopause four times greater than controls during their early twenties.[27]

Although the morbidity is less than in males, single alkylating agents (cyclophosphamide, busulfan, L-phenylalanine mustard) (RR 9.2) and MOPP are the best described culprits.[26]

There is a trend toward intensification of treatment, particularly with childhood leukemia. Thus, the risk of gonadal

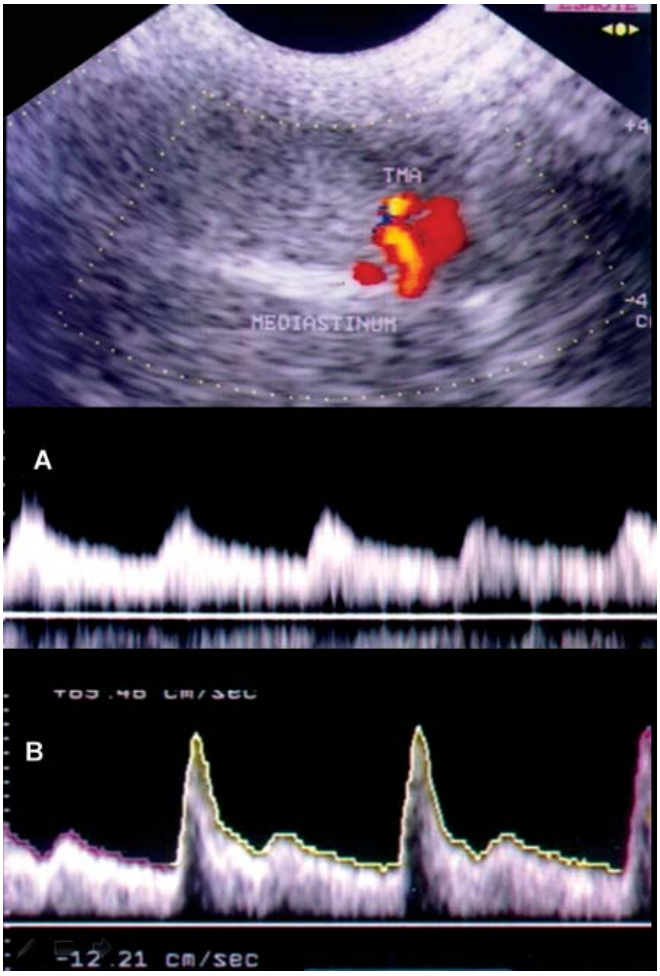

Fig. 3: Color Doppler analysis of the transmediastinal artery: (A) Normal vascularization in controls, (B) increased resistances in male survivals of childhood malignancies

damage with these evolving regimens needs to be continuously evaluated. The majority of early reports of ovarian function in children treated with combination chemotherapy for ALL suggest that premature ovarian failure (POF) is uncommon.[28] However, there have been reports of premature ovarian failure in women treated for ALL with more intensive regimens.[29] Early reports of ovarian function following the treatment of Hodgkin's disease in childhood suggested that ovarian function was normal in all women studied.[6] Children treated with cyclophosphamide (up to 200 mg/kg) as conditioning for bone marrow transplant (BMT) had normal ovarian function in 95% of cases. However, higher doses (> 200 mg/kg) result in POF.[30]

Chemotherapy does not appear to have any significant permanent adverse effect on uterine function. Successful pregnancy and healthy offspring are reported following treatment with multi-agent chemotherapy regimens.[29, 31]

In the female, radiotherapy is responsible for dose-dependent ovarian damage that may induce both sterility and impaired estradiol release. A radiation dose of 2,000 cGy below the diaphragm fractionated in 5 to 6 weeks provokes a complete depletion of the fixed pool of oocytes and ovarian failure occurs even when given in the prepubertal period.[32] Even lower radiation doses such as those used for total body irradiation before BMT (800 to 1,000 cGy), may be followed by ovarian failure in young girls. Ovarian failure (high FSH levels and low estradiol values) may be reversible, but is always responsible for a reduction in the ovary pool resulting in premature menopause.[33] Following a radiation insult, the size of the surviving population determines the "window" of opportunity for fertility and time until menopause. The depletion of primordial follicles that occurs at the time of the insult is related to the number present so that the younger the women at the time of treatment, the greater the number of follicles that survive and the later the onset of menopause. For 40 years old women, the permanent menopause is induced by 6 Gy. In younger women and children, permanent sterility is produced with a total dose of 20 Gy over 6 weeks.[34]

An additional problem is that of cranial irradiation. A 4,000 to 5,000 cGy irradiated on the hypothalamic-pituitary region result in delayed puberty and hypogonadism.[35] Cranial irradiation at lower doses (1,800 to 2,400 cGy), such as used for ALL, may be associated with early puberty and subtle perturbations in growth hormone secretion.[35] Young girls seem to be more sensitive to the cranial damage induced by irradiation, as suggested by the smaller pituitary dimension of girls treated at younger age.[36]

Recent data demonstrate that uterine function may be compromised following radiotherapy. Reduced uterine volume and decreased elasticity of uterine musculature, possibly as a consequence of impaired vascularization, are found in girls receiving pelvic, abdominal, and total body irradiation prepubertally.[37] Although, successful pregnancy following radiotherapy is reported, the incidence of spontaneous miscarriage, premature delivery and intrauterine growth retardation is significantly increased.[37] Women treated with total body irradiation who were given physiological sex steroid replacement therapy, have shown an increase in uterine volume and endometrial thickness.[38]

Investigation of Ovarian/Uterine Function

After anticancer therapy, the incidence of amenorrhea has been reported to vary between 40 and 68%.[39,40] The frequency of ovarian failure seems to depend upon the type, dose and duration of the therapy[41] and age of the patients with the eldest having a much higher incidence of permanent sterility than younger women.[39,42] At the present moment, there are no definite estimates to calculate, in relation to these parameters, the magnitude of the risk for gonadal damage.

The assessment of the ovarian reserve (i.e. basal hormonal evaluation—FSH, estradiol, inhibin B; ovarian biopsy; ultrasonographic analysis of ovarian volume and small antral

follicular count)[43] provides important information on the ovarian residual follicle pool but gives no data on spontaneous ovulation.

After ovulation, the corpus luteum forms from the dominant follicle and has a critical role in progesterone production. The rapid and massive production of progesterone is essential for regulating menses as well as for initiating and maintaining pregnancy. A mid-luteal progesterone concentration of ≥20 nmol/L is the most used indicator of an ovulatory cycle. With the advent of transvaginal probes and Doppler sonography, the corpus luteum can be now evaluated noninvasively.[44] The corpus luteum appears as an ultrasonographic highly vascularized complex structure with peripheral flow easily identified on color Doppler analysis (Fig. 4).[45]

In females, although children treated for cancer may have an increased incidence of early menopause,[42] several authors showed that a proportion of cancer survivors have regular menstrual cycle and normal endocrine profile.[46, 47] However, these patients may often experience an accelerated decline of fertility and risk involuntary childlessness which can seriously harm their self-esteem and quality of life. In the prepubertal child it is not possible to clinically or hormonally detect the potential extension of gonadal damage. However postpubertally, elevated gonadotropins levels and very low estradiol values are clear indicators of the ovarian failure and of the extensive follicular store depletion.[26] An FSH rise in early follicular phase with maintained estradiol production has been recognized to be a feature of perimenopause and is detectable years before the definitive menopause.[48,49] Nonetheless, the detection of a subtle dysfunction of the hypothalamus-pituitary-ovarian axis and the prediction of a premature menopause may be extremely difficult. Several studies observed that a significant proportion of cancer survivors who continued to have regular menses after CT/RT were at risk of developing premature menopause some years after treatment.[47,50] Therefore, reassuring clinical information does not necessary imply that the ovaries escaped the damage. In fact, Byrne *et al*[27] noted a 15% fertility deficit overall among survivors without a known reason for infertility and Larsen and coworkers[51] affirmed that the "…ovarian age of female cancer survivors frequently is higher than their chronological age…As a consequence they may have a shortened reproductive span…". Ultrasonographic measurement of ovarian volume and antral follicle counts are possible indicators of ovarian aging.[52,53]

In a recent study, despite spontaneous pubertal development, regular menstrual cycles and normal early follicular FSH levels, patients who suffered from childhood malignancies and had been treated with chemo- and radiotherapy, evidenced a mean ovarian volume lower but not statistically significant from the controls.[54] However, the cancer survivors showed increased resistances at level of intraovarian arteries (Fig. 5). From this, the authors hypothesized that the ovarian vascular

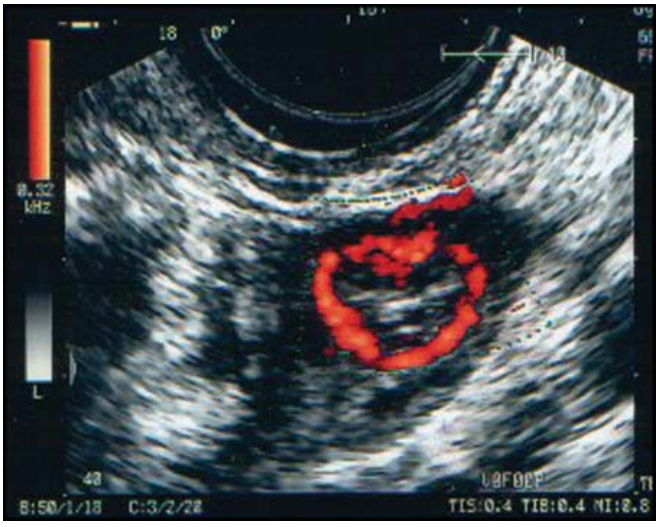

Fig. 4: Ultrasonographic representation of the corpus luteum which appears as a highly vascularized complex structure with color Doppler peripheral flow

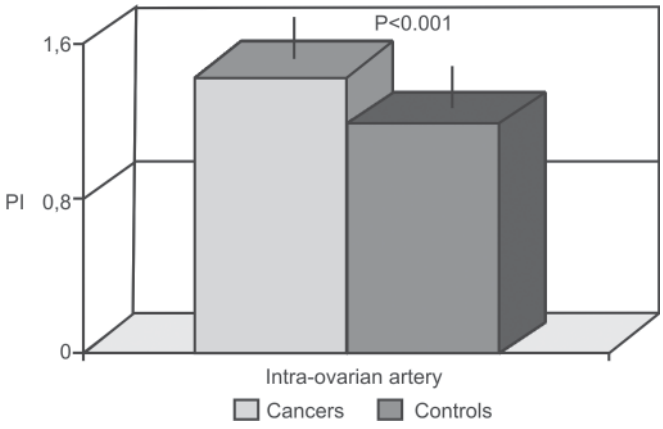

Fig. 5: Increased resistances at the level of intraovarian artery in female survivors of childhood malignancies compared to controls

deficit may represent the first alteration of ovarian function.[54] Moreover, some of the patients showed ovulatory progesterone values and a highly vascularized corpus luteum; others, despite menstruating regularly, showed no sign indicating that ovulation had occurred (Fig. 6). These nonovulatory patients had low uterine volume and the resistances registered at level of uterine arteries were the highest.[54] The authors noticed that the only difference between the ovulatory and the non-ovulatory cancer survivors was that the time elapsed between the diagnosis of cancer and menarche was longer in ovulatory patients. Thus, the authors speculated that the resistance of prepubertal ovary is not total, that the utero-ovarian structures could be more vulnerable to CT in proximity of menarche and that, even in presence of normal menstrual cycles and early

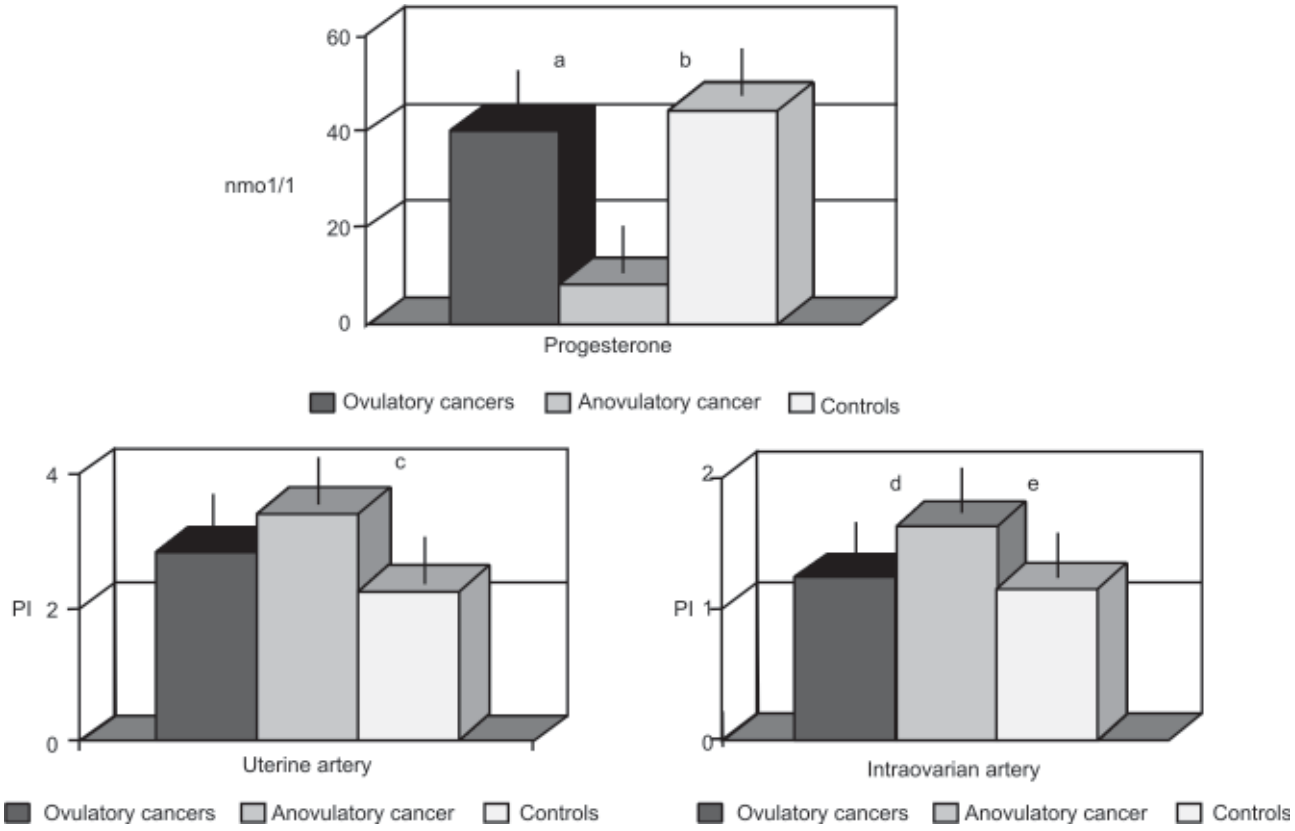

Fig. 6: Upper panel: progesterone circulating value in ovulatory and anovulatory female survivors of childhood malignancies compared to controls. Lower left panel: pulsatility index of uterine artery . Lower right panel: pulsatiliy index of intraovarian artery. Significance: [a]p < 0.0001; [b]p< 0.0001; [c]p <0.001; [d]p = 0.002; [e]p > 0.001

follicular FSH values, subtle ovarian vascular alterations may occur with increased risks of ovarian failure.[54]

FERTILITY PRESERVATION

The gonadal toxicities listed above, although not life-threatening, are of serious concern to patients and their families. Advances in assisted reproduction and increasing interest in gamete extraction and maturation have focused on preserving gonadal tissue from children before sterilizing chemo- or radiotherapy, with the realistic expectation that future technologies will be able to utilize their immature gametes.

Males

Potential strategies for preservation of male fertility are dependent upon the sexual maturation of the patient. Spermatogenesis begins in puberty, although a number of immature spermatogenic cells are described in the prepubertal testis. Spermarche is a mid-pubertal event preceding the ability to produce an ejaculate, which occurs at median age of 13.4 (11.7 to 15.2) years when median testicular volume is 11.5 ml (4.7 to 19.6 ml).[55]

Cryopreservation of Gametes

It is the only established current clinical option for preservation of male infertility. Spermatozoa are obtained from the ejaculate by masturbation but may be also obtained using rectal electrostimulation techniques under anesthetics.[56] When it is not possible to obtain an ejaculate, sperm can be retrieved by epididymal aspiration or testicular biopsy in sexually mature men.[56] Not infrequently, sperm produced by cancer patients at the time of diagnosis is of poor quality.[56] However, with the introduction of intracytoplasmatic sperm injection (ICSI), the problem of low numbers and poor motility sperm has been partially bypassed. More recently, a small number of pregnancies have been achieved with ICSI using immature spermatids and secondary spermatocytes extracted from testicular tissue in men with spermatogenic arrest.[57]

Experimental Strategies

Studies have shown that the *administration of GnRH agonists or antagonists* to rats, either immediately or after a delay following treatment with CT or irradiation, restores the ability of spermatogonia to differentiate and resume normal spermatogenesis. However, studies in humans have failed any convincing evidence to clinical benefit.[58] It is possible that endocrine manipulation to enhance recovery may be successful in patients in whom testicular damage is less severe and there is some preservation of spermatogonial stem cells.

For prepubertal boys, there are no options currently available to preserve fertility. *Harvesting testicular tissue* is an option still entirely experimental, although extremely attractive.[59] Testicular tissue could be removed before the start of treatment and cryopreserved either as a segment of tissue or as isolated germ cells. After the treatment for cancer, the frozen tissue could be thawed and autografted to the testis, or the germ cells could be matured *in vitro* until they become sufficiently mature to achieve fertilization through ICSI.

Other experimental strategies include germ cell isolation and storage, germ cell enrichment, cryopreservation of spermatogonial stem cells, germ cell transplantation, germ cell *in vitro* maturation.[19]

Females

Reducing the radiation dose to the ovary by shielding or removing the ovaries from the field of radiation (oophoropexy) may preserve ovarian function.[60] The ovaries are laparoscopically transposed to a position behind the uterus, away from the field of radiation to minimize exposure.[60] However, even when ovarian function is preserved, the uterus may be damaged and the ability to achieve and carrying to term a pregnancy may be compromised.

The *cryopreservation of embryos or oocytes* are the only current available strategy for preservation of female fertility. However, embryo banking requires the patients to have a partner or to use sperm donor. Cryopreservation of oocytes is the alternative possibility for single women without a partner, but it is much less successful than embryo banking.[19]

Experimental Strategies

It has been hypothesized that inducing a prepubertal milieu during chemotherapy would decrease the risk of POF,[19] and it has been demonstrated in rodents that GnRH analogs inhibit chemotherapy-induced ovarian follicular depletion, although this is still not an option in humans.[19]

Another experimental strategy is the prevention of follicle atresia by inhibiting apoptosis with ceramide.[19] The treatment of mice oocytes with ceramide (and its derived sphingosine-I-phosphate) prevents apoptosis *in vitro*. *In vivo*, it confers a resistance to radiation-induced apoptosis in mice, with pregnancy rates of 100%.[19]

The only option potentially available for prepubertal children and the majority of young women is the cryopreservation of ovarian tissue.[19] Ovarian tissue may be harvested by slicing the ovarian cortex which is rich in primordial follicles, or by cryopreserving the immature oocytes. Survival rates and viability post-thawing are good whether primordial follicles are stored as slices or in isolation.[19] Moreover, with reimplantation, the return of ovarian activity has been achieved; however, no pregnancies have been reported. Immature oocytes show poor survival and increased rates of chromosomal abnormalities and spindle malformations.[19]

CONCLUSIONS

During the past three decades, major improvements have been made in the treatment and cure of certain hematological malignancies, as well as solid tumors in young patients. As a result of improved survival, attention has been turned to the long-term physical and psychological sequelae of treatment. The loss of fertility and premature menopause are unfortunately important and common long-term side effects of curative radio- and chemotherapy. These facts have high impact on self-esteem and quality of life of these patients, because many are young and have expectations of a normal reproductive life. The prospect of ovarian failure in females and impaired fertility in males after antineoplastic therapy is a difficult topic for patients and clinicians to deal with, because of the lack of good prognostic information. The frequency of fertility failure varies with the type, dose, duration of radio- and chemotherapy, and age of patient. Currently, there are no good estimates of the magnitude of the risk involved in relation to these factors. It is, therefore, of outmost importance that research in this field is pursued and increased, in order to provide the appropriate information, consultation, potential preventive acts before initiation of the anticancer therapy, and hopefully, the restoration of fertility after treatment.

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