FERTILITY PRESERVATION

ADVANCES AND CONTROVERSIES

EDITORS

MOHAMED A BEDAIWY

BOTROS RMB RIZK

Foreword

Victor Gomel

JAYPEE

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4 Ovarian Reserve Testing for Cancer Patients

Luciano G Nardo, Lamiya Mohiyiddeen

CHAPTER OUTLINE

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INTRODUCTION

Women undergoing cancer treatment are at greater risk of ovarian failure and subsequent infertility. Both the age of the woman and the type and dose of the chemo-radiotherapy influence the progression to ovarian failure. The gonadotoxic effect of chemotherapeutic agents is well-documented. Alkylating agents such as cyclophosphamide that are non-cell cycle specific are more cytotoxic to the ovaries than cell cycle-specific agents such as methotrexate and fluorouracil, whose major effect is on ovarian follicle growth and maturation[1]. Ovarian damage following chemotherapy can present with a variety of symptoms that reflect varying degrees of damage, culminating in premature ovarian failure (POF).

Those patients who are not immediately rendered infertile are still likely to suffer with infertility and develop POF. Approximately, 50% of patients under age 35 resume normal menses after completion of cytotoxic chemotherapy, and are therefore potentially capable of becoming pregnant spontaneously or through assisted conception treatment.[2] Although a regular menstrual cycle may serve as a convenient marker for satisfactory ovarian function, a normal menstrual cycle is not synonymous with satisfactory fertility. Similarly, irregular menses or amenorrhea does not always imply infertility. Because of the possibility of spontaneous recovery, it has been suggested that ovarian function be reassessed periodically in patients with chemotherapy-induced gonadal damage.[3]

Bone marrow transplantation (BMT, with its associated treatments) carries the worst prognosis, even when it is performed at a very young age. Only 19% of children treated with BMT have normal ovarian function, whereas in older women ovarian failure is virtually universal.[4] Pelvic irradiation by itself has significant consequences. It has been estimated that a total radiation exposure of 20 Gy fractionated over six weeks in younger women and children produces sterility with 95% confidence.[5]

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So far, long-term follow-up studies have assessed ovarian function after cancer treatment using parameters such as follicle stimulating hormone (FSH) serum levels or clinical end-points such as cycle length or pregnancy rates. Despite the presence of high FSH levels and amenorrhea after chemotherapy, pregnancies in childhood cancer survivors have been reported as well. Hence, FSH levels or menstrual cyclicity after cancer treatment do not seem to be predictive of the chance to conceive.[6] A recent report on gonadal function in childhood cancer survivors showed that in patients with regular menstrual cycles following treatment with chemotherapy and cranial radiotherapy, ovarian reserve was compromised when assessed by serum anti-Müllerian hormone (AMH) concentrations.[7] Serum AMH levels, antral follicle count (AFC) and ovarian volume are more reliable predictors of ovarian function in cancer patients compared to FSH levels.

The increasing number of young survivors of cancer with favorable outcomes is defining the need for a more comprehensive approach that will improve the quality of life after cancer, including the preservation of fertility.

EFFECT OF CHEMOTHERAPY ON THE OVARY

The fact that ovaries of young girls contain a higher number of follicles than ovaries from older women makes them more resistant to chemotherapy. Thus, young girls tolerate higher doses of chemotherapy before entering POF. However, chemo-therapeutic agents differ in relation to their toxicity on ovarian function. Cyclophosphamide and busulfan, which have frequently been used in the treatment of childhood cancer, are far more gonadotoxic than other agents [1]. Chemotherapy disrupts ovarian function by depleting the primordial follicle pool in a drug- and dose-dependent manner. Analysis of ovarian function after cancer therapy have mostly described the prevalence of ovarian failure after treatment, although follicular depletion may occur despite maintenance of regular menstrual cycles.

EFFECT OF RADIOTHERAPY ON THE OVARY

Despite the fact that radiotherapy interrupts the normal cellular proliferation cycle, causing extensive cell damage and that postnatal oocytes are mitotically inactive, they are still highly susceptible to the damage caused by radiotherapy. This is probably because of the risk of undergoing induced apoptosis. The high number of follicles in the pre-pubertal ovary makes it less vulnerable than the ovary of women in late reproductive life. That said the risk of primary ovarian failure after abdominal radiotherapy is still high. Complete ovarian failure occurs with a dose of 20 Gy in women under 40 years of age and with only 6 Gy in older women.[8] The uterus is extremely vulnerable to irradiation and decreases in volume by 40%. It has been suggested that if pregnancy occurs, problems may ensue that are related to abnormalities in uterine function.

ROLE OF OVARIAN RESERVE TESTS (ORT) IN CANCER PATIENTS

The natural fecundity and pregnancy rates decrease with increasing age, however, it is established that chronological age and menstrual characteristics are unreliable in predicting reproductive age. Several models have now been described that accurately chart the progressive decline of primordial follicles in the ovary with the woman's chronological age. The most important aspect of diminished ovarian reserve and the associated decline in reproductive potential is that its onset is highly variable. The continual decline in follicle number and quality is a dynamic process.

Treatment with chemo-therapeutic agents may not only have an immediate effect, but may cause a significant reduction in the ovarian reserve in the long term. Ovarian reserve markers can help identify the women who are at risk of this. These patients can be advised at an early age against postponing childbearing too long, as their reproductive career may be shorter than they expect.

The ability to predict a woman's reproductive potential would be of considerable value for many young cancer survivors for their treatment decision-making, family planning and contraception concerns. Women interested in future fertility should be able to undergo ovarian reserve testing before and upon completion of cancer therapy.

OVARIAN RESERVE TESTS

Follicle Stimulating Hormone

Biochemical markers like basal serum FSH is considered an indirect estimate of ovarian reserve and depends on the presence of an intact hypothalamus-pituitary-ovarian axis. It is one of the most established parameters for estimating ovarian performance. The values of FSH usually maintain within normal ranges and tend to rise only when ovarian function is deeply compromised.

Some studies suggest that young age exerts a protective effect over the deleterious effects of reduced ovarian reserve, while others reveal no difference in outcome between younger and older women with elevated FSH. Threshold levels for baseline FSH are also an issue. In one study, which is unique for its duration of follow-up, ongoing pregnancy was seen in as much as 28% of regularly cycling women with FSH levels of 15–20 IU/L. Only when the FSH level was >20 IU/L was there a clear fall in ongoing pregnancy rate regardless of age.[9] Others have shown elevated FSH to be of limited value in patients with regular cycles. Questions remain regarding the reproducibility of FSH measurements. One of its main limitations is the significant intercycle variability of FSH, hence limiting the usefulness of a normal value.

Most of the data regarding the performance of FSH in predicting ovarian reserve comes from studies in *in vitro* fertilization (IVF) populations. Information pertaining to the general sub-fertile population and cancer patients is lacking, but a case–control study revealed no statistically significant difference

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in cumulative pregnancy rates between patients with elevated FSH levels and controls. On day 3 of the menstrual cycle, serum FSH levels are usually less than 10 IU/L in most assays. FSH levels that are more than 15 IU/L on day 3 suggest a decreased ovarian reserve and a reduced probability of pregnancy; if values exceed 20 IU/L, the probability of pregnancy is extremely low.[10]

The establishment of threshold values within individual assay systems is also important to prevent errors in interpretation of results. This is more likely to happen in institutions where the clinical volume of patients is insufficient to allow these threshold values to be established. Other reasons for an elevated baseline FSH should be considered. Laboratory errors can occur in the presence of heterophilic antibodies, which can interfere with the FSH immunoassay.[10] FSH receptor variants have now been identified.[11] This may cause slightly diminished receptor function, resulting in higher levels of FSH to achieve adequate receptor response, whilst not necessarily affecting ovarian reserve.

Although basal FSH remains a frequently used marker of ovarian reserve in any level of fertility care, the controversies surrounding its use as a predictor of ovarian reserve and the fact that FSH is not a direct reflection of selectable ovarian follicles makes it a less reliable marker.

Anti-Müllerian Hormone

Serum AMH has emerged as a valuable marker of ovarian reserve in women of reproductive age. AMH is secreted by ovarian granulosa cells of primordial, preantral and small antral follicles. After puberty and before perimenopause, the AMH level remains stable in healthy women. Serum levels of AMH represent the quantity of the ovarian follicle pool, whilst the relationship with the quality has not yet been confirmed. AMH was found to be a more sensitive predictor of ovarian reserve than other markers.[12] AMH is relatively convenient to determine, especially as it seems to exhibit a relatively stable expression during the menstrual cycle, making it an attractive determinant of ovarian reserve.

A study in cancer patients showed that AMH levels were lower in the patients compared to controls, even though the latter group was older. This indicates that ovarian reserve in patients with cancer was already compromised before any therapy was initiated. Remarkably, all patients who developed POF after gonadotoxic chemotherapy had pre-treatment AMH levels lower than the 10th percentile of AMH levels in age-matched controls. However, not all patients with pre-treatment AMH below this threshold point developed POF. In this study, AMH levels prior to treatment were not predictive for ovarian reserve after treatment.[13]

Following chemotherapy, the decrease in AMH levels per year was not significantly different from that of controls. This suggests that ovarian damage after gonadotoxic therapy is due to a diminished primordial follicle pool rather than to an increased rate of follicle loss.

In a study of childhood cancer survivors including 185 patients, serum AMH levels were significantly lower in survivors who received radiotherapy of the abdomen, pelvis, sacrum or their total body than in controls, whereas survivors irradiated on other parts of their body had normal AMH levels.[7] There were no differences in AMH levels in subgroups classified according to disease. Survivors treated with three or more procarbazine containing chemotherapy cycles had significantly lower AMH levels than controls. The decrease in AMH levels was noted to precede changes in conventional parameters associated with perimenopausal status, such as FSH, inhibin B and estradiol.

Recently, AMH was measured in survivors of childhood Hodgkin's lymphoma, hematological malignancies and solid tumors. These studies confirmed that, in childhood cancer survivors with regular menstrual cycles, serum AMH levels were indicative of limited ovarian reserve, whereas FSH, estradiol and inhibin B levels were not different from healthy controls.[14,15] It may be premature to apply a cut-off value since this has not been validated in the population under study.

Although the secretory function of the ovary may recover to some extent and menses may return after the completion of chemotherapy treatment, ovarian reserve remains persistently affected. Neither baseline nor change in AMH predicts return of menstrual function, suggesting that ovarian reserve and endocrine function may be affected differently or may recover differently from chemotherapy.[7]

Antral Follicle Count

AFC is usually defined as the number of follicles measuring between 2–10mm in diameter detected by ultrasound scan in the early follicular phase. Studies in women with proven fertility reveal a continual decline of AFC with age. Additionally, AFC shows a better correlation with chronological age than other hormonal and ultrasound markers.

Reproducibility has been addressed and described as being moderate, with decreasing agreement at higher AFC. Intercycle variability has also been described as moderate in a fertile population. A more recent analysis, which studied infertile patients, showed that intercycle variability was greater than interobserver variations, suggesting a biological variation of AFC from cycle to cycle. AFC < 5 is predictive of poor ovarian reserve.[16]

A lower level of AMH appears to be the best serum predictor of diminished AFC, which is thought to reflect reduced likelihood of future pregnancy. Because AMH is a serologic test that does not fluctuate with the menstrual cycle, assessment of AMH may be a more practical factor for the measurement of fertility potential than AFC, which requires transvaginal scan.

Inhibin B

Inhibins are secreted by the ovarian granulosa and luteal cells during the menstrual cycle. Inhibins are a part of the hypothalamus-pituitary-ovarian axis, specifically having an inhibitory effect on pituitary FSH synthesis and secretion. Serum inhibin B is regarded as a direct measure of ovarian reserve as it is mainly secreted by antral follicles. Inhibin B rises from early in the follicular phase to reach a peak coincident with the onset of the mid-follicular phase decline in FSH concentrations. It then declines during the luteal phase apart from a periovular peak.

Inhibin B has also been shown to respond to exogenous FSH administration in the follicular phase. Studies in older women reveal significantly lower levels of inhibin B in women aged 40–50 years with raised FSH compared to women with

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normal FSH. Concentrations of inhibin B have been shown to be lower in women with elevated FSH and regular menstrual cycles. Decreased inhibin B was found in women with normal FSH levels, implying that decreased inhibin B precedes a rise in FSH.[17] Inhibin B has been evaluated in predicting response as a component of dynamic testing. These include the clomiphene citrate challenge test (CCCT), GnRH agonist test (GAST) and the exogenous FSH ovarian reserve test (EFORT). In a prospective study, the inhibin B response to exogenous FSH correlated well with AFC and the number of oocytes retrieved.[18] Given the expense of performing the test, further prospective evaluation and confirmation of its predictive capacity is required before inhibin B can assume a routine place in the clinical assessment of women with reduced ovarian reserve.

Ovarian Volume

The human ovary is a dynamic organ which changes in size and activity throughout its lifetime. Mean ovarian volume increases from 0.7 cm^3 at age 10 years to 5.8 cm^3 at age 17 years. Estimation of the mean primordial follicle population in women aged 25–51 years can be made using the Faddy–Gosden equation[19] and correlating it with mean ovarian volume at each chronological age.[20] This model makes a critical assumption that primordial follicle populations are fixed at birth, a notion that has recently been challenged. Whether or not ovarian volume can accurately reflect primordial follicle numbers or indeed follicle quality is questionable. The latter may be more important if reproductive age is to be correlated with fertility outcome. Ovarian volume estimation using conventional ultrasound is inexpensive and relatively easy to perform. Intra- and inter-observer variations are small, ensuring reproducibility. Its main limitation at present is the lack of data regarding ovarian volume measurements in the general population, both fertile and infertile, across different age cohorts.

Estradiol (E2)

The condensed follicular phase length in older women may be as a result of a more advanced follicular recruitment by cycle day 3. This early dominant follicle selection is expressed by high serum E2 concentrations. It has been shown in an assisted reproduction treatment population (where GnRH analogs were not administered) that increasing day 3 estradiol concentrations are associated with decreasing oocyte numbers and pregnancy rates. The predictive ability of E2 is improved in patients of advanced reproductive age, especially when combined with FSH.[21] No data are currently available regarding basal E2 levels in the general population. Further studies including data on day 3 estradiol values and fecundity in spontaneous cycles are required before evaluating this parameter further in cancer patients.

Dynamic Ovarian Reserve Tests

The recognized limitations of biochemical markers of ovarian reserve led to the development of a number of dynamic ovarian tests in an attempt to 'unmask' patients whose diminished ovarian reserve may have been missed using these markers.

The GAST (or G-test) evaluates the E2 serum concentration change from cycle day 2 to day 3 after administration of a GnRH agonist, the latter causing a temporary increase in pituitary secretion of gonadotropins. Early studies were able to show a limited ability for the early rise in E2 concentrations to differentiate between normal and reduced ovarian reserve, with different threshold values. Inhibin B is considered a direct marker of ovarian reserve. Combining Inhibin B with the GAST may have improved the predictive value of the test.[22] The GAST appears to have much promise as a dynamic test of ovarian reserve, but has not yet been validated outside an assisted reproductive setting. Furthermore, the cost of performing the test has limited its acceptability.

The CCCT involves the administration of 100 mg clomiphene citrate on cycle days 5–9 and the determination of FSH concentrations on days 3 and 10. In women with normal ovarian reserve, the overall increase in E2 and inhibin production by the developing follicles should be able to overcome the estrogen antagonist effect of clomiphene on the hypothalamus-pituitary-ovarian axis and suppress FSH levels back into the normal range by day 10. The CCCT may be more indicative of oocyte quality than quantity. Nevertheless, a quantitative relationship between the CCCT and follicle density has been shown.[23] Correlation with other biophysical markers of ovarian reserve (ovarian volume and AFC) has been shown in an infertile population.

The EFORT combines FSH with the rise in E2 over a 24 hour period after administration of a standardized dose (300 IU) of purified FSH on day 3. A randomized, prospective study in which basal and dynamic tests of ovarian reserve were compared revealed the EFORT as being the best predictor of ovarian reserve.[24] Further prospective evaluation is required to validate this test properly.

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Hormonal Suppression

Gonadotropin releasing hormone analogs (GnRH-a) have been utilized during chemotherapy to suppress ovarian cycling and induce a temporary medical menopause. The action of GnRH-as are not clearly understood because primordial and primary follicles do not have GnRH receptors and it is possible that GnRH-a preserve those follicles that have already initiated growth. It has also been speculated that the hypo-estrogenic state caused by GnRH-a may decrease utero-ovarian perfusion, resulting in a decreased total cumulative exposure of the ovaries to the chemotherapeutic insult. Several studies in animals have suggested that protection may be offered by use of GnRH-a, however, the benefit in human therapy remains debatable.

A recent prospective, randomized, controlled study was published analyzing a group of 80 breast cancer patients younger than 40 years of age. The cancer patients were randomized to either co-treatment with GnRH-a (Zoladex, 3.6 mg subcutaneously) during chemotherapy (study group, n = 39) or treated with chemotherapy alone (control group, n = 39), with one dropout in each group.[25] Eighty-nine percent in the study group resumed

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menses and 69% resumed spontaneous ovulation, as compared with 33.3% and 25.6%, respectively, in the control group (P < 0.01). Median FSH and luteinizing hormone (LH) concentrations were significantly lower in the study group compared with the control group (P <0.01), suggesting a better ovarian reserve in the study group.

A recent trial of 281 patients suggested that the use of triptorelin-induced temporary ovarian suppression during chemotherapy in pre-menopausal patients with early-stage breast cancer reduced the occurrence of chemotherapy-induced early menopause.[26] Collectively, the few available studies suggest that there may be some degree of protection with GnRH-a. Before puberty, concomitant use of GnRH-a are not recommended. The human studies presently available are limited in design and lack long-term follow-up.

Transposition of the Ovaries (also known as Oophoropexy)

The aim of this approach is to surgically remove the ovaries from the direct field of radiation. The technique was first applied to women with cervical cancer in the 1950s. In many clinics, oophoropexy is now standard procedure in women suffering from cancers that require pelvic irradiation. Although the ovaries may be out of the field of direct radiation, the scatter dose may cause significant ovarian damage. In a review, it was found that women receiving pelvic irradiation after oophoropexy still experienced ovarian failure in 50–90% of the cases.[27] Complications to the procedure include chronic pelvic pain, vascular injury, fallopian tube infarction and ovarian migration—although all occurred rarely. Another aspect to be considered is the accessibility of the ovaries for oocyte collection if IVF becomes necessary. It is very likely that a transposed ovary located in the paracolic gutter may be inaccessible for safe oocyte retrieval.

Oophoropexy could be considered in cases of abdominal irradiation, either unilaterally or bilaterally, but the long-term effect remains uncertain. It may protect the ovarian function in <50% of cases in adults, but further clinical data providing good evidence for this approach is required.

Cryopreservation of Oocytes

The first report of a live birth originating from human cryopreserved oocytes appeared in 1986. The technique involves stimulation of the ovaries and harvesting of eggs followed by freezing. Stored eggs can later be thawed and IVF techniques with intracytoplasmic sperm injection (ICSI) can be employed. Oocytes are much more sensitive to damage from cryopreservation than embryos (probably secondary to spindle damage from ice crystal formation). The formation of ice crystal and the attendant cellular damage during freezing can potentially be avoided by vitrification.

An alternative approach could be the collection of immature oocytes that are subsequently put through *in vitro* maturation (IVM). When using this technique, oocytes can be collected at any time during the cycle (stimulated or unstimulated), or can even be retrieved from excised ovarian tissue in conjunction with cryopreservation of gonadal tissue. Since the first report of a birth from a frozen oocyte, the results of this procedure worldwide have been variable with a reported success rate of 2% per oocyte retrieved, despite the improved success rate when combined with ICSI.

Cryopreservation of Embryos

This is a very well-established technique in cancer patients. It involves controlled ovarian stimulation using exogenous gonadotropins, generating multiple follicular development so that several oocytes can be collected. This technique requires 2–4 weeks for completion and therefore can delay the antineoplastic treatment. In cancer patients, the fresh embryos are not replaced and are cryopreserved for later use. Recently, a protocol has been described in which ovarian stimulation may start even during the luteal phase in cancer patients, thus requiring a maximum of 2 weeks before oocyte collection. The stimulation of the ovaries using gonadotropins results in high estrogen levels, and certainly this raises concerns for some steroid sensitive tumors. It is still unclear what the risks of such techniques are in terms of tumor progression or relapse in a hormone dependent cancer. This risk has been addressed by using tamoxifen or letrozole alone or in combination with standard IVF stimulation for women with breast cancer or endometrial cancer. This is an established method available in clinical practice to preserve fertility in women being treated for cancer.

Cryopreservation of Ovarian Tissue

This is a technique that can be used for adults and for children, but at present it is still regarded as experimental due to the lack of large longitudinal follow up studies. An entire ovary, or part of it, is removed surgically, often during laparoscopy or in conjunction with an operation for the disease. The cortex, which is the site of the primordial follicles, is isolated 1–2 mm in thickness and cut into strips or fragments. The tissue can be kept indefinitely in liquid nitrogen at –196°C. After the patient is cured, some of the cryopreserved tissue can be transplanted back in to the pelvis, and follicular growth, endogenous hormone production and ovulation may resume. Optimal treatment benefit can only be expected in the presence of a healthy ovarian reserve. As such, it is less likely to be beneficial for older women and those with a suboptimal ovarian performance. Donnez and colleagues reported in 2004 a live birth after transplanting cryopreserved ovarian tissue back into the pelvis of a woman following treatment for stage 4 non-Hodgkin's lymphoma.[28] All the cases of successful post-implantation pregnancy reported to date have utilized the whole cortical ovarian tissue. The risk of re-implanting tissue with occult cancer, while small, remains a matter of concern and ongoing research. Only patients with cancer associated with low risk of ovarian metastasis, such as squamous cell carcinoma of the cervix, Wilm's tumor, Hodgkin and non-Hodgkin's lymphoma, should be considered for future auto-transplantation. On the contrary, women with moderate and high risk of ovarian primary or secondary involvement should not be offered this fertility preservation option.

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CONCLUSION

Chemotherapy decreases ovarian reserve rapidly and significantly. Although the secretory function of the ovary may recover to some extent and menses may return after completion of chemotherapy treatment, ovarian reserve remains persistently affected. Chronological age remains an important factor for the recovery of endocrine function after chemotherapy and potentially for fertility. For young women looking for fertility preservation before the start of chemotherapy, the most reliable and successful method to date is embryo freezing, although other options can be offered. Oocytes or ovarian tissue cryopreservation and GnRH agonist or antagonist therapy in conjunction with chemotherapy are all active areas of research. Oktay and colleagues reported using a combined letrozole-FSH protocol for ovulation induction in cancer patients that produced results comparable to those of standard IVF without a significant increase in E2 levels.[29]

Some studies have shown that female cancer survivors with regular menses and normal serum FSH levels had significantly smaller ovaries along with a lower AFC as compared to age-matched controls. These observations indicate that parameters such as cycle length and FSH serum levels are not accurate markers of ovarian function. Serum AMH levels, AFC and ovarian volume appear to be more reliable predictors. Neither baseline nor change in AMH concentrations was seen to predict return of menstrual function, suggesting that ovarian reserve and endocrine function may be affected differently or may recover differently from chemotherapy.

The relationship between AMH and other markers of ovarian reserve has been assessed, where AMH was found to be a more sensitive predictor of ovarian reserve than the other markers. AMH is relatively convenient to determine, especially as it seems to exhibit a relatively stable expression during the menstrual cycle, making it an attractive determinant of ovarian reserve. More prospective large studies are required to validate ovarian reserve markers in cancer patients in order to determine cut off levels to accurately predict the possible reproductive outcome in this cohort of women.

REFERENCES

1. Hensley ML, Reichman BS. Fertility and pregnancy after adjuvant chemotherapy for breast cancer. Crit Rev Oncol Hematol. 1998; 28:121-8.
2. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1997. CA Cancer J Clin. 1997;47:5-27.
3. Singh KL, Davies M, Chatterjee R. Fertility in female cancer survivors: Pathophysiology preservation and the role of ovarian reserve testing. Hum Reprod Update. 2005;11:69-89.
4. Barrett A, Nicholls J, Gibson B. Late effects of total body irradiation. Radiother Oncol. 1987;9:131-5.
5. Locatelli F, Giorgiani G, Pession A, Bozzola M. Late effects in children after bone marrow transplantation: a review. Haematologica. 1993;78:319-28.
6. Sklar C. Maintenance of ovarian function and risk of premature menopause related to cancer treatment. J Natl Cancer Inst Monogr. 2005;34:25-7.
7. Fong SL, Laven JSE, Hakvoort-Cammel FGAJ, et al. Assessment of ovarian reserve in adult childhood cancer survivors using anti-Mullerian hormone. Hum Reprod. 2009;24:982-90.
8. Wallace WHB, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. Int J Radiat Oncol Biol Phys. 2005;62:738-44.
9. van Rooij IA, de Jong E, Broekmans FJ, et al. High follicle-stimulating hormone levels should not necessarily lead to the exclusion of subfertile patients from treatment. Fertil Steril. 2004;81:1478-85.
10. Scott RT Jr. Diminished ovarian reserve and access to care. Fertil Steril. 2004;81:1489-92.
11. Perez MM, Gromoll J, Behre HM, et al. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab. 2000; 85:3365-69.
12. Nardo LG, Gelbaya TA, Wilkinson H, et al. Circulating basal anti-Müllerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for in vitro fertilization. Fertil Steril. 2009;92:1586-93.
13. Lie Fong S, Lugtenburg PJ, Schipper I, et al. Anti-mullerian hormone as a marker of ovarian function in women after chemotherapy and radiotherapy for haematological malignancies. Hum Reprod. 2008;23:674-8.
14. Bath LE, Wallace WH, Shaw MP, et al. Depletion of ovarian reserve in young women after treatment for cancer in childhood: detection by anti-Mullerian hormone, inhibin B and ovarian ultrasound. Hum Reprod. 2003;18:2368-74.
15. van Beek RD, van den Heuvel Eibrink MM, Laven JSE, et al. Anti-Mullerian hormone is a sensitive serum marker for gonadal function in women treated for Hodgkin's lymphoma during childhood. J Clin Endocrinol Metab. 2007;92:3869-74.
16. Su HI, Chung K, Sammel MD, et al. antral follicle count provides additive information to hormone measures for determining ovarian function in breast cancer survivors. Fertil Steril. 2011;95:1857-59.
17. de Koning CH, Popp-Snijders C, Schoemaker J, et al. Elevated FSH concentrations in imminent ovarian failure are associated with higher FSH and LH pulse amplitude, response to GnRH. Hum Reprod. 2000;15:1452-56.
18. Yong PY, Baird DT, Thong KJ, et al. Prospective analysis of the relationships between the ovarian follicle cohort and basal FSH concentration, the inhibin response to exogenous FSH and ovarian follicle number at different stages of the normal menstrual cycle and after pituitary down-regulation. Hum Reprod. 2003;18:35-44.
19. Faddy MJ, Gosden RG. A model conforming the decline in follicle numbers to the age of menopause in women. Hum Reprod. 1996;11:1484-86.
20. Wallace WH, Kelsey TW. Ovarian reserve and reproductive age may be determined from measurement of ovarian volume by transvaginal sonography. Hum Reprod. 2004;19:1612-17.
21. Buyalos RP, Daneshmand S, Brzechffa PR. Basal estradiol and follicle-stimulating hormone predict fecundity in women of advanced reproductive age undergoing ovulation induction therapy. Fertil Steril. 1997;68:272-7.
22. Ravhon A, Lavery S, Michael S, et al. Dynamic assays of inhibin B and oestradiol following buserelin acetate administration as predictors of ovarian response in IVF. Hum Reprod. 2000;15:2297-301.
23. Hofmann GE, Sosnowski J, Scott RT, et al. Efficacy of selection criteria for ovarian reserve screening using the clomiphene citrate challenge test in a tertiary fertility center population. Fertil Steril. 1996;66:49-53.
24. Kwee J, Elting MW, Schats R, et al. Comparison of endocrine tests with respect to their predictive value on the outcome of

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ovarian hyperstimulation in IVF treatment: results of a prospective randomized study. Hum Reprod. 2003;18:1422–27.
25. Badawy A, Elnashar A, El-Ashry M, et al. Gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage: prospective randomized study. Fertil Steril. 2009;91:694–7.
26. Mastro D, Boni L, Michelotti L, et al. Effect of the gonadotropin-releasing hormone analogue triptorelin on the occurrence of chemotherapy-induced early menopause in premenopausal women with breast cancer: a randomized trial. JAMA 2011;3:312-4.
27. Wo JY, Viswanathan AN. Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients. Int J Radiat Oncol Biol Phys. 2009;73:1304-12.
28. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364:1405-10.
29. Oktay K, Buyuk E, Libertella N, et al. Fertility Preservation in Breast Cancer Patients: A Prospective Controlled Comparison of Ovarian Stimulation with Tamoxifen and Letrozole for Embryo Cryopreservation. Journ of Clin Oncol. 2005;23:4347-53.

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Mohamed A Bedaiwy MD PhD FACOG is Professor and Division Head of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada. He is board-certified by the American Board of Obstetrics and Gynecology. He received his medical degree from Assiut University School of Medicine, Egypt, where he graduated Valedictorian, Summa Cum Laude. He completed a residency in Obstetrics and Gynecology in 1997. He received his doctorate degree in 2003 through a combined program with Cleveland Clinic Foundation (CCF)/Lerner College of Medicine/Case Western Reserve University (CWRU), Ohio, USA. Moreover, he completed an American Board certified residency in Obstetrics and Gynecology and a fellowship in Reproductive Endocrinology and Infertility at the University Hospital Case Medical Center, CWRU, USA. He also completed a minimally invasive surgery clinical fellowship at the Cleveland Clinic Foundation. In addition, he completed a clinical fellowship in Reproductive Endocrinology and Infertility in the Division of Reproductive Sciences, University of Toronto, Canada. He was awarded a PhD degree from the University of Maastricht, the Netherlands, for his work on ovarian tissue cryopreservation and transplantation. He has a well-established international reputation as a scholar. He is a prolific author with over 200 publications, including 104 peer-reviewed publications. He is best known for his work in endometriosis, ovarian transplantation, and minimally invasive surgery. His interests include assisted reproduction, minimally invasive surgery, robotic surgery, fertility preservation in cancer patients, and endometriosis.



Botros RMB Rizk MD MA FRCOG FRCS HCLD FACOG FACS is the Professor and Head, Reproductive Endocrinology and Infertility, and Medical and Scientific Director of USA In Vitro Fertilization Program and Assisted Reproduction, University of South Alabama, Mobile, Alabama, USA. Mentored by Nobel laureate Professor Robert Edwards, who was awarded the Nobel prize in Medicine for achieving the first successful in vitro fertilization in the world, Dr Rizk started his career in Reproductive Medicine in London and Cambridge, UK, where most of the current scientific developments and medical protocols were invented. He joined the prestigious University of Cambridge for three years from 1990 to 1993. He is board-certified by the American Board of Obstetrics and Gynecology, the Royal College of Physicians and Surgeons of Canada and the Royal College of Obstetricians and Gynecologists in England, UK, as well as, the American Board of Bioanalysts in Embryology and Andrology.

Dr Rizk's areas of clinical and research interests have been ovarian stimulation including the development of the long agonist protocol inspired by his colleagues Professors Howard Jacobs, Seang Lin Tan and Charles Kingsland. He is internationally considered as a leading authority on ovarian hyperstimulation syndrome (OHSS) and made original contributions to the pathophysiology and prevention of the syndrome. He authored a book solely dedicated to ovarian hyperstimulation syndrome that is considered the standard reference for OHSS. He is an authority on endometriosis; its novel medical management and robotic surgery.

Dr Rizk published 12 medical textbooks embracing different aspects of infertility and assisted reproduction, as well as ultrasonography and robotic surgery. He is one of the early members of European Society for Human Reproduction and a founding member of Middle East Fertility Society. He chaired successfully for several years the American Society for Reproductive Medicine (ASRM) postgraduate courses on ART. He is the President Elect of the Middle East Fertility Society.

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