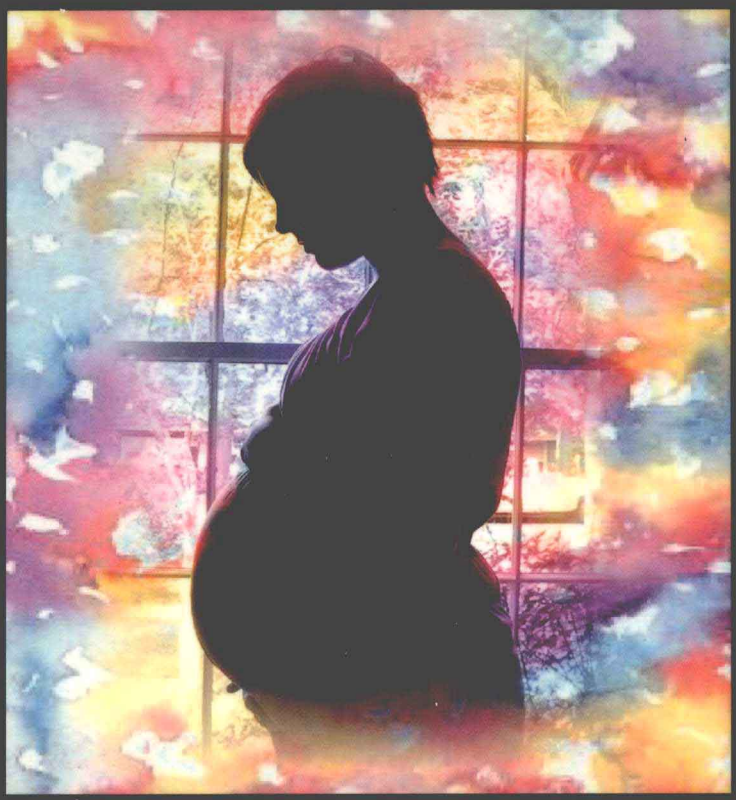
Fertility Research Trends



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Editors

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Preface

Chapter I - With the improvements in anticancer treatment, the survival rate of adolescent cancer victims is increasing. As a consequence of this therapy, many female adolescents suffer from ovarian failure and premature ovarian failure. The level of infertility varies by patient age (the younger the patient, the lesser the likehood of severe ovarian failure) as well as by drug dose, treatment duration, type of treatment (alkylating agents cause severe ovarian damage), and number of agents used. The highest risk is associated with high-dose chemotherapy combined with total body irradiation before bone marrow transplantation. Ovarian damage caused by irradiation is also correlated to the anatomical location of the ovaries in relation to the radiation field in the abdomen. Unfortunately, cancer patients have limited options for fertility preservation. Most survivors require oocyte donation. However, there is a shortage of donated oocytes in Western countries on the one hand, and most women prefer fertility restoration with self-oocytes on the other. Of the feasible possibilities for fertility preservation from self-oocytes, embryo cryopreservation after in vitro fertilization (IVF), as well as cryopreservation of mature human oocytes, are in most cases unsuitable for minors. Therefore, cryopreservation of ovarian tissue containing small immature ovarian follicles remains almost the only practical option. So far, the autologous transplantation of cryopreserved-thawed ovarian tissue in women has resulted in four pregnancies, including two live births. Attempts to establish an in vitro maturation system are still in their infancy. This technology followed by routine IVF would eliminate the risks of reseeding malignant cells by ovarian grafts, and could possibly be offered in the future to infertile survivors of adolescent cancer, particularly of hematological malignancies. Obtaining ovarian tissue for cryopreservation requires a surgical procedure, usually laparoscopy, under general anesthesia, which may harbor risks that need to be discussed with the patient and her guardians. Other issues, such as the optimal surgical technique and the amount of tissue that needs to be removed for successful cryopreservation, have yet to be resolved. Although fertility-restoration techniques are still in the experimental stage, ovarian tissue removed from prepubertal and adolescent cancer patients is being frozen-stored in some medical centers worldwide. Sustaining the option of parenthood for young female patients may convey a message of hope, with a positive psychological effect.

Chapter II - Many cultures consider reproduction to be of great importance, an obligatory achievement expected of any established couple. Infertility therefore may result in stress to a

couple trying to conceive. This couple will undoubtedly experience feelings of frustration and disappointment if a pregnancy is not easily achieved. Labeled as having a fertility problem may result in a severe insult to self-esteem, body image, and self assessed masculinity or femininity. Despite the fact that various studies have demonstrated the importance of the mind-body connection and fertility, the psychosocial aspects of infertility have not been adequately addressed. The present review was aimed to determine the connection between psychological stress and both male and female infertility. Several studies addressing the psychological aspects of infertility will be discussed in this chapter, as well as evidence of infertility related to stress. More has been written regarding female stress and infertility and less was published about this evident body-mind connection regarding males. Psychological factors are risk factors of subsequent infertility among women. Moreover, the experience of the diagnosis and treatment of infertility causes subsequent psychological distress. A clear connection between stress and female infertility emerges from reading the presented research done in this field. It yet remains unclear as to what extent this connection is a predictor of failure or success, is it different among male and female individuals and what are the best intervention methods to improve fertility and psychological outcome. The knowledge existing today regarding the influence of emotional factors on male fertility is limited. Psychological stress, in addition to being a result of infertility problems, may also be a cause for decreased fertility.

Further studies including intervention programs should be conducted in order to evaluate the true impact of stress upon male fertility. Meanwhile, stress parameters should be an important part of history taking and an integral parameter in regular follow up of patients diagnosed or treated for infertility.

Chapter III - Hospitals, care providers, insurance companies, and baby food manufacturers are among the many groups interested in the number of newborns. The U.S. Census Bureau uses information on changing childbearing patterns to help project the number of people who will be living in the United States in the future.

In the early 1900s, women averaged about four children during their childbearing years, while those living during the Great Depression averaged about two. After World War II, the total fertility rate for women climbed to 3.7 by 1957, then fell to 1.8 by the mid-1970s. During the past decade, the total fertility rate has fluctuated between 2.0 and 2.1—just below the level required for natural replacement of the population.

In June 2004, 61.6 million women aged 15 to 44 lived in the United States, according to the June Fertility Supplement to the Current Population Survey (CPS). During the preceding 12 months, 3.7 million of these women gave birth—resulting in a fertility rate of 61 births per 1000 women. First-time mothers accounted for 1.5 million of these births—producing a first-birth rate of 24 births per 1,000 women.

Among women aged 40 to 44 in 2004, 19 percent were childless—twice the percentage of women this same age who were childless in 1976. Women approached the end of their childbearing years with an average of 1.9 children in 2004, compared with 3.1 in 1976.

Chapter IV - What happens to a woman who conceptualizes her gender and her fertility in a certain way when she moves from one social, cultural and political environment to another, characterized by disparate social constructions of gender and fertility? What factors encourage her to keep the previously structured identity? What factors force her into a re-

construction or acculturation of her gender role and, therefore, a different conception of her fertility? How does she negotiate these double expectations? What support does she need in order to be successful in that process?

These questions, identified through the authors community work, were the focus of the research project "Immigrant Women and Fertility: Rights and Responsibilities". The authors conducted the research to complete the academic requirements for the Master of Arts degree at the Department of Anthropology at the University of Alberta, but also to fulfill the obligations to Planned Parenthood Edmonton, the organization with which she is affiliated and that adopted the project and obtained funding from the Status of Women Canada. In that light, the research project brought together all four pillars of the competent community-based research: identified community need, the professional principles of research conduct, the relevant, client oriented non-profit organization that stood behind the project, and the sufficient financial support.

Chapter V - The paper investigates the demographic alternatives for dealing with the projected population aging and low or negative growth of the population and labor force in the North. Without further immigration, the total labor force in Europe and Russia, the high-income countries of East Asia and the Pacific, China, and, to a lesser extent, North America is projected to be reduced by 29 million by 2025 and by 244 million by 2050. In contrast, the labor force in the South is projected to add some 1.55 billion, predominantly in South and Central Asia and in Sub-Saharan Africa. The demographic policy scenarios to deal with the projected shrinking of the labor forth in the North include moving the total fertility rate back to replacement levels, increasing labor force participation of the existing population through a variety of measures, and filling the demographic gaps through enhanced immigration. The estimations indicate that each of these policy scenarios may partially or even fully compensate for the projected labor force gap by 2050. But a review of the policy measures to make these demographic scenarios happen also suggests that governments may not be able to initiate or accommodate the required change.

Chapter VI - Since 1950 Spain has shown two parallel trends of dramatic drops in fertility and in religiosity (secularization). This paper explores the relationship between secularization and fertility among Spanish Catholics. The authors use a unique, rich, data set which includes various dimensions of religiosity: respondent's religious affiliation; current church attendance (six levels); current prayer habits (eleven levels); spouse's religious affiliation; parental religious affiliation; and parental (maternal and paternal) and respondent's church attendance during childhood (nine levels). The multi-facet data on religiosity (rather than a single dichotomous variable) allow for a sophisticated analysis, permitting rigorous conclusions to be drawn. The sample is restricted to married Catholic (female and male) respondents who were raised by Catholic parents and are married to a Catholic spouse in order to obtain a homogenous sample and to focus on the effect of the level (intensity) of religiosity (rather than religious affiliation) on fertility. Fertility is related to the various dimensions of religiosity; first using cross-tabulation and then using OLS regression.

The authors' results are substantive: i) They find that fertility is not related to the current intensity of religiosity. ii) Exposure to religious activities during childhood has a significant effect on women's fertility (but not men). Interestingly, a father who rarely attended church services has a negative effect on his daughter's future fertility (decreasing the number of

children by about 0.8), while the mother's inactive churchgoing has an unexpected positive effect (leading to a increase of one child). iii) The respondent's own church attendance during childhood does not have any effect on current fertility.

In sum, this study demonstrates the significance of childhood experience in shaping one's 'taste for children'. It also suggests that there is no direct link between the rapid process of secularization occurring in Spain and the decline in birth rates.

Chapter VII - The development of an effective egg cryopreservation system will have a significant impact on the clinical practice of assisted reproduction and prevent egg aging. In addition to fertility preservation for young women requiring sterilizing medical or surgical treatments and those who are at risk of premature ovarian failure, cryobanking of eggs can benefit a large population of women who wish to delay motherhood because of personal, professional and financial reasons. Donor egg programs can also benefit from an efficient egg cryopreservation protocol, eliminating the need for donor-recipient cycle synchronization. Egg freezing procedure is minimally invasive and avoids the ethical and moral concerns related to cryopreservation of embryos. The main drawbacks of conventional slow-cooling/rapid thawing protocols for egg cryopreservation have been low survival and pregnancy rates, resulting in approximately 114 human live births over the last quarter of century. Recent development in vitrification or ultra-rapid-freezing of eggs appears more promising as it is faster, simpler and cheaper than the conventional slow-cooling method. Vitrification involves extremely high cooling and warming rates (>20,000°C/min), resulting in glasslike solidification without the formation of ice crystals during the cooling and warming processes. Vitrification of eggs is associated with improved survival, embryonic development and pregnancy rates. Within the past seven years, more than 32 human live births have been reported. In summary, vitrification of eggs is novel method to preserve women's fertility and unique technology to prevent egg aging.

Chapter VIII - Although tobacco smoking is a widely recognized health hazard and a major cause of preventable mortality, smoking remains prevalent in our society. Approximately 30% of women and 35% of men of reproductive age smoke. But more importantly, the incidence of smoking is increasing among adolescents and specifically teenage girls. Environmental tobacco smoke (ETS), or passive smoke, is a significant source of exposure to a large number of substances known to be hazardous to human health. Recently, substantial harmful effects of cigarette smoke on the ability to become pregnant have become apparent. The deleterious effect of passive smoke exposure is similar to active smoking with respect to fertility. Women who smoke take longer to achieve a natural pregnancy, require more hormonal stimulation during IVF treatment with reduced success, and reach menopause earlier than their non-smoking counterparts. Toxicants found in passive smoke have been isolated at higher levels in the serum and follicular fluid of women exposed to ETS compared to non-smokers implying that cigarette smoke may act as an ovarian toxicant as one mechanism to disrupt fertility. However, the male contribution to infertility cannot be excluded. The paternal effect on infertility is largely dependant on sperm function. The effect of cotinine, for example, has been shown to alter sperm function, and may be an underlying mechanism of impaired fertility. Paternal smoking is associated with sperm with a higher incidence of DNA abnormalities that may account for higher implantation failures and miscarriage rates. Both the female and male gamete are sensitive to toxic exposure(s) and

slight alterations to either the sperm or oocyte may be lethal at the peri-implantation stage of embryo development leading to sub-fertility. Passive smoke exposure may induce toxic effects and compromise fertility through several direct or indirect mechanisms.

Chapter IX - The effects of circannual changes in photoperiod and environment on the reproductive performance in the pig are reviewed. The ancestral pig is a short day seasonal breeder and therefore seasonal effects on fertility of the pig may be regarded as physiological by nature. In principle, the breeding season in the pig is thought to be controlled by the central nervous system, synchronized by the ambient photoperiod and further modified by other environmental cues such as availability of food and ambient temperature. As in other short day seasonal breeders, the breeding season in the pig is favoured in midwinter in order to provide the offspring with best chances to survive in the following spring four months later. Under hard circumstances when food is difficult to find and temperatures fall too far below the thermal comfort zone, the breeding season of the pig may be delayed. In the modern pig industry, several parameters of reproductive performance indicate seasonal fluctuation in reproductive efficiency corresponding well to the seasonality of breeding seen in the wild for this species. Within the industry, this phenomenon is known as seasonal infertility and is characterised by lower pregnancy rates, lower proportion of females in oestrus when expected and delayed puberty when the pigs are kept in long days. Seasonal infertility is seen as an indicator period of the year, revealing the level of reproductive management and stress in a herd. Low pregnancy rates are likely due to a seasonal disruption of pregnancy, which is economically important and may be caused by a hormonal mechanism. Central effects of the pineal melatonin may suppress LH pulsatility and thereby reduce the pituitary support for CL, retarding the embryonic development and interfering with the physiological mechanism for establishment of pregnancy. Detrimental seasonal effects on reproduction may be avoided by adequate lighting programs involving alternation of short and long days, by providing female pigs with abundant feed, by using breed records for genetic selection, by using cooling systems where the upper thermal comfort zone is exceeded and by providing the pig with adequate shelter, bedding material and freedom to move under cold conditions.

Contents

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Chapter I

Fertility Preservation in Female Adolescents with Malignancies

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Abstract

With the improvements in anticancer treatment, the survival rate of adolescent cancer victims is increasing. As a consequence of this therapy, many female adolescents suffer from ovarian failure and premature ovarian failure. The level of infertility varies by patient age (the younger the patient, the lesser the likehood of severe ovarian failure) as well as by drug dose, treatment duration, type of treatment (alkylating agents cause severe ovarian damage), and number of agents used. The highest risk is associated with high-dose chemotherapy combined with total body irradiation before bone marrow transplantation. Ovarian damage caused by irradiation is also correlated to the anatomical location of the ovaries in relation to the radiation field in the abdomen. Unfortunately, cancer patients have limited options for fertility preservation. Most survivors require oocyte donation. However, there is a shortage of donated oocytes in Western countries on the one hand, and most women prefer fertility restoration with self-oocytes on the other. Of the feasible possibilities for fertility preservation from self-oocytes, embryo cryopreservation after in vitro fertilization (IVF) as well as cryopreservation of mature

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human oocytes are in most cases unsuitable for minors. Therefore, cryopreservation of ovarian tissue containing small immature ovarian follicles remains almost the only practical option. So far, the autologous transplantation of cryopreserved-thawed ovarian tissue in women has resulted in four pregnancies, including two live births. Attempts to establish an *in vitro* maturation system are still in their infancy. This technology followed by routine IVF would eliminate the risks of reseeding malignant cells by ovarian grafts, and could possibly be offered in the future to infertile survivors of adolescent cancer, particularly of hematological malignancies. Obtaining ovarian tissue for cryopreservation requires a surgical procedure, usually laparoscopy, under general anesthesia, which may harbor risks that need to be discussed with the patient and her guardians. Other issues, such as the optimal surgical technique and the amount of tissue that needs to be removed for successful cryopreservation, have yet to be resolved. Although fertility-restoration techniques are still in the experimental stage, ovarian tissue removed from prepubertal and adolescent cancer patients is being frozen-stored in some medical centers worldwide. Sustaining the option of parenthood for young female patients may convey a message of hope, with a positive psychological effect.

Introduction

Relevant Aspects of Human Oogenesis, Folliculogensis and Early Pregnancy

In this section, the *in vivo* development of germ cells and follicles from fetal to adult life and the early stages of pregnancy will be discussed briefly, focusing on topics essential for the understanding of later sections [Speroff *et al.*, 1994; Abir *et al.*, 2006].

The female is born with a complete pool of germ cells (oocytes). Their number declines gradually from birth (about one million) to menopause (average age of 51), mostly by atresia but also by ovulations [Faddy and Gosden, 1996].

Human primordial germ cells (PGC) arrive from the yolk sac to the gonad from day 26 of pregnancy, and are then termed oogonia [Gosden, 1995; Abir *et al.*, 2006]. Thereafter, three events induce the development of the female fetal germ cells in the gonad (ovary): mitotic division cycles of the oogonia; meiotic division; and follicular assembly. The number of female germ cells in the fetal ovary peaks at about seven million in mid-pregnancy, and then drops dramatically during the third trimester. Meiotic division usually commences gradually in the third month of gestation, and the diplothen stage is achieved within weeks of its initiation. At this point, the oogonia increase in size and acquire more intracellular organelles and the nucleus becomes round and large: *germinal vesicle (GV).* These germ cells are now termed oocytes. Just before birth, the oocytes are arrested at the diplothen stage of the prophase of the first meiotic division. They have completed genetic recombination and do not undergo any additional nuclear maturation until puberty when menstrual cycles are initiated.

Follicular formation in humans begins during the fourth month of gestation. During follicular assembly, there is a rapid proliferation of the nearby cells, and the oocytes become surrounded by a single layer of flattened somatic cells, termed granulosa cells (GC) [Gougeon, 1996; Abir *et al.*, 2006]. These cellular complexes, called *primordial follicles*, measure 30-50 μm in diameter, and can be identified in humans from around 22 gestational

weeks. Most of the follicles in human fetal as well as in adult ovaries remain in the quiescent primordial form. Their growth regulation is not hormonal, and the exact factors that stimulate their development are unknown [Abir *et al.*, 2006]. Primordial follicles are activated when their GC become cuboidal, and they are then termed *primary follicles* (50-80 μm in diameter).

Primary follicles develop to *secondary follicles* (80 μm -0.2 mm in diameter) with an increased proliferation rate of the GC and consequent formation of a multilaminar layer. In humans, a definitive *theca layer* is created from the stroma cells surrounding the secondary follicle. Steroid hormones are synthesized through complex interactions between the GC and theca cells. During the secondary follicular stage, the oocyte starts to grow, and a glycoprotein coat, the *zona pellucida (ZP)*, forms between the oocyte and the innermost layer of the GC. The final follicular stage consists of development of *antral follicles* (early antral follicle: 0.2-0.4 mm in diameter) containing a fluid-filled cavity within several layers of cuboidal GC; the innermost layers surrounding the oocytes are termed *cumulus cells*.

At puberty, the hypothalamic gonadotrophin releasing hormone (GnRH) is secreted in pulsatile discharges and stimulates the normal production of the pituitary gonadotrophins: follicle stimulating hormone (FSH) and lutenizing hormone (LH) [Speroff *et al.*, 1994]. FSH levels rise in the first half of the menstrual cycle before ovulation, inducing follicular development and growth from secondary stages (the *follicular phase*) [Speroff *et al.*, 1994; Abir *et al.*, 2006]. A primordial follicle reaches the ovulatory antral size (18-20 mm in diameter) within six to nine months [Gougeon, 1996]. Ovulation occurs at the mid menstrual cycle and is stimulated by a drastic increase in baseline LH levels (*LH surge*) [Speroff *et al.*, 1994]. During ovulation, the first meiotic division is completed, with the extrusion of the first polar body enclosed between the oocyte and the ZP, forming a mature oocyte. The ovarian site from which ovulation occurs, develops into a progesterone-secreting gland, the *corpus luteum*. The remaining days until the end of the cycle are termed the *luteal phase*.

The ovarian phases are directly associated with changes in the uterine endometrium. The increased estrogen secretion during the follicular phase stimulates endometrial proliferation until a final preovulatory thickness of over 8 mm is achieved. After ovulation, endometrial development is affected by fluctuations in progesterone and estrogen levels, and is characterized by the appearance of glandular cells, coiling of spiral blood vessels, and expression of sex steroid receptors on the endometrial blood vessels. Implantation occurs on days 21-22 of the cycle, followed by the formation and proliferation of the decidual cells from the endometrium on day 23, under the influence of progesterone. The decidual layer is essential for the invasiveness of the embryonic trophoblast required for normal implantation.

Recently, studies have reported the presence proliferating PGC in ovaries of adult mice [Johnson *et al.*, 2004; 2005]. Although PGC have also been identified in the bone marrow (BM) of mice [Johnson *et al.*, 2005], ovarian function in humans has not been restored after BM transplantation (BMT) for cancer therapy (see next section). The presence of these cells in human adult ovaries is not only controversial but also highly unlikely, taking menopause into account [Faddy and Gosden, 1996; Gougeon, 1996]. Moreover, although some authors observed that PGC developed in cultures of human surface ovarian cells [Bukovski *et al.*, 2004; 2005], others failed to identify them in ovaries from women aged 28-53 years [Liu *et al.*, 2007].

Fertility in Survivors of Childhood or Adolescent Cancer

General Aspects of Childhood and Adolescent Malignancies

Childhood cancer is relatively rare with an annual incidence of around 110 to 130 cases per one million children [Stiller, 2004]; and 1 in every 600 children will develop cancer in the first 15 years of life [Bath *et al.*, 2002]. The estimated relative frequencies of pediatric cancers are as follows: leukemias, 34% (acute lymphoblastic leukemia-ALL ~one third; acute myeloid leukemia-AML ~20%) [Leung *et al.*, 2000; Brougham and Wallace, 2005]; brain/spinal cancer, 24% (central nervous system-CNS tumors are the second most common solid cancers in childhood) [Wallace *et al.*, 2005a]; embryonic cancer, 15%; lymphomas, 11% (Hodgkin lymphoma-HL is the most common solid tumor in adolescents); soft tissue cancer, 6%; bone cancer, 5%; and others, 5% [Leung *et al.*, 2000; Brougham and Wallace, 2005; Wallace *et al.*, 2005a]. Breast cancer is rare in adolescence, with an incidence of <0.1 per 100000 individuals <20 years of age, accounting for <1% of all childhood cancers. Its most common form, secretory cancer, occurs more frequently in children than in adults [Shannon and Smith, 2003].

Major advances in anticancer treatment in recent decades, particularly the use of multi-agent chemotherapeutic regiments, have resulted in a significant increase in the survival rates of children and adolescents to 70%-80% [Linet *et al.*, 1999; Mertens *et al.*, 2001; Brougham and Wallace, 2005; Sklar *et al.*, 2006]. The current survival rate for various forms of leukemia is at least 70% [Hann *et al.*, 2000; Bath *et al.*, 2002]. Today 1 in every 100 adults in their third decade is a survivor of childhood cancer, and by 2010, an estimated 1 in every 715 adults in the general population will be a survivor of childhood or adolescent cancer [Bath *et al.*, 2002]. With the rise in the number of survivors, some long-term side effects of anticancer treatment have become apparent, and their potential effect on quality of life. One of these is fertility problems which in most cases are detected only later in life [Bath *et al.*, 2002; Brougham and Wallace, 2005; Wallace *et al.*, 2005a].

General Aspects of Subfertility after Anticancer Treatment

The potential adverse effects of anticancer treatment on reproductive function in female survivors may be mediated through the ovary [Bath *et al.*, 2002], the uterus [Bath *et al.*, 1999; 2002], or the hypothalamus-pituitary-ovarian axis [Bath *et al.*, 2001; 2002]. Specifically, systemic chemotherapy or spinal, abdominal, or pelvic radiotherapy can accelerate oocyte depletion and lead to various degrees of ovarian failure [Meirow and Nugent, 2001; Brougham and Wallace, 2005].

Chemotherapeutic agents affect dividing cells, and therefore, the mechanism by which they damage the quiescent primordial follicles is unknown [Meirow, 2000; Meirow and Nugent, 2001]. Although the common assumption is that they do so by initiating apoptosis [Tilly, 1996], limited evidence has been provided so far by only two *in vitro* studies [Perez *et al.*, 1997; Meirow, 2000; Meirow and Nugent, 2001]. Indeed, investigations initiated in our laboratory did not identify any traces of apoptosis in postchemotherapy ovarian follicles of patients aged 6-39 (mean: 17 ± 10 years) (Figure 1). This was also true at 4 days after treatment onset in a 14-year-old patient [Abir *et al.*, 2008]. However, we did find a significant decrease in follicular quality, as indicated by the reduction in normal GC nuclei

and concomitant increase in oocyte vacuolization (Figure 2). Given that follicles cannot remain viable without normal interactions between the oocytes and the GC [Gougeon, 1996], the follicular destruction after chemotherapy could occur in response to changes in the GC. Although large numbers of follicles were identified in ovaries of girls ≤20 years old after therapy, it is possible that a portion of them will eventually deteriorate because of poor quality, especially of the GC [Abir *et al.*, 2008].

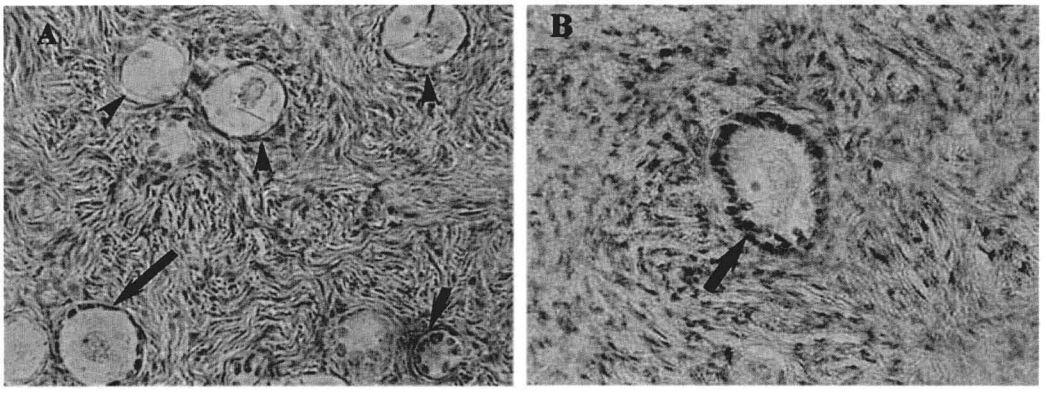

Figure 1. Photographs showing outcome of staining to detect apoptosis. (A) Ovarian section from a 15-year-old patient with HL after chemotherapy that included cyclophosphamide. Note the primordial (arrowheads) and primary follicles (arrows), the exclusively methyl green staining, with lack of brown staining for apoptosis. Original magnification, X400. (B) Positive control of an ovarian section treated with DNase from a 17-year-old patient with HL after chemotherapy that included cyclophosphamide. Note the primary follicle (arrow), and the brown staining indicating apoptosis, in addition to the background methyl green staining. Original magnification, X400.

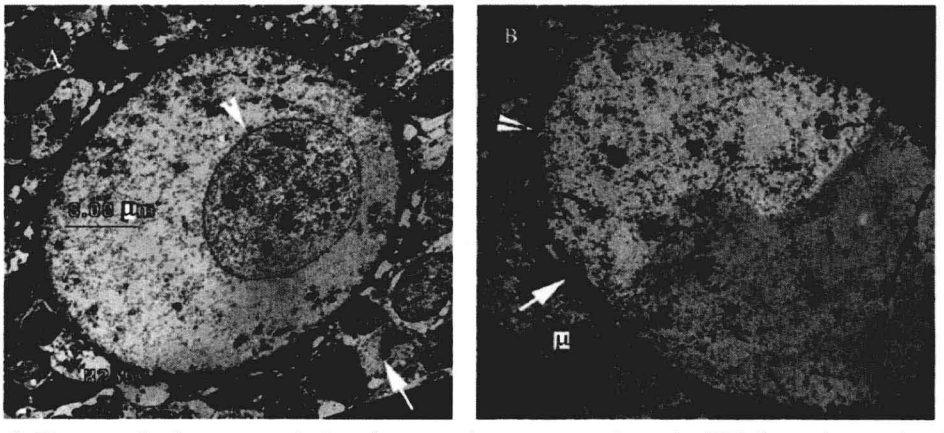

Figure 2. Photographs from transmission electron microscopy sections. A. (TEM) ovarian section from a 13-year-old patient before anticancer treatment. Note the normal secondary follicle with its GV (arrowhead) and normal GC layer (arrow). Original magnification, X2500. (B) TEM ovarian section from the same patient as in figure 1 (B). Note the primordial follicle with its damaged GC layer and nuclei (arrow) and basement membrane (arrowhead). Original magnification, X4000.

The level of ovarian damage varies by patient age at the time of treatment and the chemotherapy agents used [Meirow and Nugent, 2001; Abir *et al.*, 2007]. Regarding age, young girls, especially at prepubertal ages, appear to be relatively protected against

chemotherapy-induced ovarian damage, probably because of their larger reserve of surviving primordial follicles [Meirow and Nugent, 2001; Brougham and Wallace, 2005; Abir *et al.*, 2007]. Some studies also attributed this finding to the premenarcheal hormonal status (Marcello *et al.*, 1990; Abir *et al.*, 1998), although our results do not confirm this association [Abir *et al.*, 2008].

Regarding the treatment protocol, the integrity of the ovaries is affected by the type of chemotherapeutic agents, the cumulative dose, the total dose, and the duration of the treatment [Abir *et al.*, 1998]. The risks of ovarian damage associated with the various preparations are summarized in Table 1 [Grundy *et al.*, 2001; Meirow and Nugent, 2001; Wallace *et al.*, 2005a; Lee *et al.*, 2006]. In general, alkylating agents pose the highest risk of infertility, although the risk is equally high for certain nonalkylating agents such as porcarbazine [Meirow and Nugent, 2001; Wallace *et al.*, 2005a]. It rises even further when alkylating agents are combined with high levels of abdominal-pelvic radiation [Wallace *et al.*, 2005a]. The pre-BMT conditioning protocol of busulfan and cyclophosphamide leads to high sterilization rates, whereas high-dose melfalan seems safer [Meirow and Nugent, 2001; Wallace *et al.*, 2005a]. Nevertheless, when a pre-BMT conditioning regimen that included cyclophosphamide was used in children, only 5% had ovarian failure, emphasizing the importance of young age as an ovarian protector [Bath *et al.*, 2002]. Pre-BMT total body irradiation (TBI), and high doses of chemotherapeutic agents were found to create a high risk of severe ovarian dysfunction [Abir *et al.*, 1998; Meirow, 2000; Meirow and Nugent, 2001; Wallace *et al.*, 2005a].

Table 1. Risk levels for infertility promoted by various chemotherapeutic agents

Risk level	Agents
High	Alkylating agents: cyclophosphamide, ifosfamide, chlormethine, busulfan, melphalan, chlorambucil, carmustine, lomustine, mechlorethamine; procarbazide
Medium	Cisplatin, carboplatin, doxorubicin
Low	Vincristine, methotrexate, dactinomycin, bleomycin, mercaptopurine, vinca alkaloids (vinblastine), fluorouracil
Risk exists-unknown level	Nitrosources & antimetabolites: cytosine arabinoside
Unknown risk	Taxanes, oxaliplatin, monoclonal antibodies (trastuzumab, bevacizumab, cetuximab), tyrosine kinase inhibitors (eriotinib, imatinib)

Modified from Grundy *et al.*, 2001; Wallace *et al.*, 2005a; Lee *et al.*, 2006.

Effects of Radiation Therapy on the Ovary

TBI before BMT, and abdominal or pelvic radiation can by itself also result in ovarian damage. The degree of impairment depends on the radiation dose, location of the ovaries relative to the radiation field, fractionation schedule, and age at treatment [Abir *et al.*, 1998; Brougham and Wallace, 2005]. Even scattered radiation may cause ovarian damage [Abir *et al.*, 1998]. The extent of oocyte depletion after radiotherapy depends on the size of the existing oocyte pool. Therefore, the younger the irradiated patient, the later the onset of premature ovarian failure (POF) [Brougham and Wallace, 2005].

The human oocyte is very sensitive to radiation and the lethal dose required for the destruction of 50% of the oocytes is ~<2 Grey (Gy) [Wallace *et al.*, 2003; Brougham and Wallace, 2005]. Childhood TBI at 10-15.75 Gy can result in 90% POF of various degrees [Bath *et al.*, 1999; Brougham and Wallace, 2005; Wallace *et al.*, 2005b; Edgar and Wallace, 2007], while 20-30 Gy can lead to 97% ovarian failure [Edgar and Wallace, 2007]. However, one study reported that 56% of girls treated prepubertally with TBI had spontaneous puberty [Sarafogolu *et al.*, 1997], and those in whom ovarian failure developed were older at the time of radiotherapy (8.6 vs 6.1 years). Pregnancies have also been reported in patients who were treated with TBI in childhood/adolescence [Sanders *et al.*, 1996; Bath *et al*, 2002].

Effects of Radiation Therapy on the Uterus

TBI and abdominal or pelvic radiation at doses of 14-30 Gy in children and adolescents can often disrupt uterine anatomy and function (receptivity and maintenance of pregnancy) [Sanders *et al.*, 1996; Bath *et al.*, 1999; 2002; Brougham and Wallace, 2005; Edgar and Wallace, 2007]. This might be a result of diminished radiotherapy-induced elasticity of the uterine musculature, uterine fibrosis, and vascular damage, possibly associated with cervical incompetence as well. Uterine growth at puberty is stimulated by ovarian estrogen production. Therefore, the final uterine volume after radiotherapy depends on the age and pubertal status of the patient at the time of treatment [Critchley and Wallace, 2005; Signorello *et al.*, 2006; Edgar and Wallace, 2007]. The risk of uterine damage is probably greater in girls treated before menarche. Uterine irradiation was found to reduce uterine volume in 100 survivors of childhood cancer [Larsen *et al.*, 2004; Edgar and Wallace, 2007].

Unfortunately, current medical technologies are limited and uterine changes can be only partially assessed before pregnancy, and mostly only after puberty [Bath *et al.*, 2002]. Ultrasound scanning is a reliable means for evaluating uterine size, shape, blood supply, and endometrial thickness. Normal uterine sizes and volume have been well documented for prepubertal, pubertal, and menopausal patients. In addition, the uterine shape changes throughout puberty and is therefore an indicator of the pubertal stage. Using Doppler scanning clinicians can identify uterine artery blood flow and quantify it by the degree of resistance to the flow. In addition, endometrial biopsies can be easily obtained and prepared for histologic study and assays of the relevant immunohistochemical markers.

Studies of pregnancy outcome after uterine irradiation in childhood/adolescence have reported an increased incidence of nulliparity, premature birth and/or low birth weight, intrauterine growth retardation, stillbirth, and possibly, spontaneous miscarriages [Chiarelli *et al.*, 2000; Green *et al.*, 2002a; 2002b; Brougham and Wallace, 2005; Edgar and Wallace, 2007]. The recent Childhood Cancer Survivor Study (CCSS) investigated the long-term outcome of a cohort of 5-year survivors of childhood/adolescent cancer who were diagnosed before age 21 years, between 1970 and 1986 [Edgar and Wallace, 2007]. A total of 2201 singleton infants were born to 1264 survivors exposed to uterine irradiation [Signorello *et al.*, 2006; Edgar and Wallace, 2007]. There were significant risks of preterm birth (<37 weeks), low birth weight (<2500gr), and small for gestational age (SGA: $<10^{th}$ centile) babies; all of which were significantly correlated with maternal receipt of high cumulative dose of uterine radiotherapy (>50 Gy) before menarche [Edgar and Wallace, 2007].

Cranial Irradiation

Cranial or cranio-spinal radiation treatment may have adverse effects on reproductive function, mediated by the hypothalamic-pituitary-ovarian axis [Bath *et al.*, 2001; 2002]. Studies have reported disruptions in the hypothalamic–pituitary-gonadal axis in patients treated with high dose cranial or craniospinal irradiation (>23 Gy) for brain tumors, with a 60% risk of gonadotrophin deficiency by 4 years from treatment [Hall *et al.*, 1994; Bath *et al.*, 2001; 2002]. Delayed puberty, pubertal arrest, and primary amenorrhea were often documented (see next two chapters on treatment) [Bath *et al.*, 2001; Brougham and Wallace, 2005; Edgar and Wallace, 2007]. Abnormal gonadotrophin levels and delayed puberty were less common following exclusive CNS radiation [Brougham and Wallace, 2005; Chow *et al.*, 2007; Edgar and Wallace, 2007]. In contrast, elevated gonadotrophin levels associated with ovarian dysfunction were noted in many children treated with craniospinal radiation, probably as a result of the damaging effects of the chemotherapy on the ovary [Hamre *et al.*, 1987].

Given the high survival rates of childhood and adolescent ALL, more attention should be directed to the effects of low-dose cranial irradiation (18-24 Gy) often administrated to patients with CNS involved ALL or recurrent ALL [Bath *et al.*, 2001; Edgar and Wallace, 2007]. Irregularities in menarche are often reported after such treatment, although the findings are conflicting [Bath *et al.*, 2001]. One study investigated 188 survivors, all diagnosed at a mean age of 7 years, most of whom received cranial radiotherapy, but only 14% were exposed to alkylating agents [Mills *et al.*, 1997]. Significantly higher irregularities in onset of menarche were detected in the survivors treated with cranio/spinal radiation at a dose of 24 Gy or with lower doses (~18 Gy) before the age of 8. The CCSS evaluated 72 survivors of ALL, all of whom were diagnosed prior to menarche and exposed to cranio/spinal radiotherapy (15-29 Gy directed to the brain+10-24 Gy to the spine in 90%), as well as chemotherapy with alkylating agents [Chow *et al.*, 2007]. They found that cranio/spinal radiotherapy was associated with delayed menarche, and cranial radiotherapy with earlier menarche, regardless of radiation level (<20 or ≥20 Gy), compared with the patients' siblings. The risk of early menarche was positively correlated with younger age at diagnosis (<5) and radiation doses of >18 Gy. These risks for menarche irregularities were absent in patients treated with chemotherapy only.

Cranial irradiation for ALL during childhood or adolescence may also be associated with lower first-birth rate, as identified in 23 survivors of ALL who delivered 41 children [Nygaard *et al.*, 1991]. A study of gonadotrophin secretion in 12 survivors of ALL with regular menses who were treated prepubertally with cranial irradiation (10-14 Gy) as well as chemotherapy, documented lower than normal estrone secretion throughout the cycle, in addition to lower early follicular phase plasma estradiol and LH levels, particularly during the LH surge, with a shorter luteal phase (≤11 days) [Bath *et al.*, 2001]. Ovarian size, however, was normal as were FSH levels.

Cranial irradiation for treatment of ALL might also indirectly affect fertility by reducing levels of growth hormone (GH) [Bath *et al.*, 2001; 2002], leading to insufficient production of insulin like growth factor 1, to reduction in the effects of gonadotrophins on the ovary, and to inhibition in uterine receptivity [Homburg and Farhi, 1995].