FERTILITY PRESERVATION IN CLINICAL PRACTICE

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FOREWORD

The war against cancer is long and exhausting. We presently employ various medical weapons that are both efficient and effective in the long run. Successful treatment often requires compromises on the part of both doctors and patients. One such concession is the patient's permanent loss of reproductive capacity, which has frequent long-term psychological consequences. Cancer patient fertility is the subject of a new medical field called ***oncofertility***. This new medical discipline connects the world of basic scientific research with actual clinical practice. This field expands cooperation among embryologists, biologists, oncologists, sociologists, and reproductive medicine specialists. Oncofertility provides a modern, relevant practice that creates positive opportunities for young people struggling with cancer. This sinister disease, while often curable, significantly impacts a patient's future reproductive ability.

In the following chapters, readers will become informed about the basic principles of this new medical discipline. They will become aware of recent molecular biology research in terms of potential clinical applications. We will also discuss certain legal, ethical, and social aspects related to this new and fascinating knowledge.

This work defines oncofertility as a new direction in reproductive medicine. It summarizes the latest findings and tries to predict successful discoveries and further advancements in this exciting multidisciplinary field. The content contained within the following pages will both attract readers and inspire associates to become further involved in improving the quality of life of cancer patients whose future fertility has been severely compromised.

Martin Huser

CONTENT

1 ONCOFERTILITY AS A NEW FIELD OF REPRODUCTIVE MEDICINE

Martin Huser

With advances in contemporary medicine, cancer is becoming a disease that can be successfully and permanently cured. Despite the use of modern methods of cancer treatment, attempts to cure a patient can be compounded by a number of side effects with potentially permanent consequences. The permanent loss of reproductive ability, often with long-term psychological impacts on patients and their quality of life, is one of the serious long-term consequences of modern anticancer therapy.

Recently, the protection of reproductive functions and organs in oncology has become a widely discussed topic, mainly because of significant successes with cancer therapy along with strong pressure to postpone childbearing to a later age, when fertility, especially among women, decreases relatively rapidly with age. Advances in cancer treatment have improved long-term survival among young patients suffering from serious forms of this disease. Many generalized forms of cancer, including leukemia, lymphomas, and a number of solid malignant tumors, are now permanently curable. Over the past decade, the field of reproductive aging generated many new insights. This knowledge enables us to offer women procedures that can effectively maintain and protect their reproductive health (Huser et al., 2010a).

The question of preserving reproductive potential is also important for women suffering from certain chronic noncancer diseases. The ovaries are unfortunately the only nonrenewable reservoir of gonadal cells in a woman's body. The treatment of many diseases irreparably damages gonadal cell function among women, for example, the treatment of severe endometriosis after all conservative

medical and surgical treatment options have failed. Among women of childbearing age, we increasingly diagnose various forms of systemic autoimmune diseases, the most common of which is ***systemic lupus erythematosus.*** If this dangerous and progressive systemic disease begins to affect vital organs, especially the kidneys and nervous system, it is vital to apply gonadotoxic cyclophosphamide immunosuppressive therapy (Nemec et al., 2008).

1.1 THE TERM ONCOFERTILITY

Cancer patient fertility concerns are the topic of a new medical field that is increasingly referred to as oncofertility. This fitting term, which was coined in 2003 by a group of American reproductive medicine specialists headed by Prof. Teresa K. Woodruff of Chicago's Northwestern University, has gradually been accepted and is now in use worldwide. Oncofertility is still a relatively obscure expression among the Czech medical community, but we will certainly hear more about it in the future.

The concept of oncofertility may be defined as the development and clinical application of reproductive protection methods for patients with cancer. For men, the "gold standard method" is cryopreservation of sperm before cancer treatment. This procedure is commonly offered in the Czech Republic and is already well established within the consciousness of the medical community (Crha et al., 2009). However, new oncofertility techniques are being developed for women. Modern methods include embryological cryopreservation storage procedures for embryos, oocytes, and ovarian tissue. Protective applications of gonadoliberine analogs (GnRH-a) during systemic cancer treatment are also gradually penetrating clinical practice. Experimental methods include a process for the ***in vitro*** cultivation of ovarian follicles to create haploid oocytes in the laboratory that are ready for fertilization. Research is frequently carried out in animal models, including mice, rabbits, and monkeys, and is also performed on human ovarian tissue.

1.2 REPRODUCTIVE PROTECTION CENTERS

In recent years, new specialized clinics called reproductive protection centers have been established in clinical practice. The main purpose of these clinics is to provide consulting services using the above methods for patients with newly diagnosed malignancy. These centers also engage in research activities in the context of developing experimental possibilities for reproductive protection. This research includes procedures such as the ***in vitro*** cultivation of ovarian follicles, the vitrification of oocytes or ovarian tissue, and ovarian tissue xenotransplantation. Experimental procedures subsequently involve participation in international consortia and professional associations, such as the ***International Society for Fertility Preservation*** *(ISFP)* (*www.isfp-fertility.org*) or the ***Oncofertility Consortium*** (*www.oncofertility.northwestern.edu*).

In 2009, a Reproductive Protection Center was formally founded in the Gynecology and Obstetrics Department of Brno University Hospital and Masaryk University Medical School in Brno. This center has offered services to patients from all over the Czech Republic and has recently offered services to patients from Slovakia as well. An important task for experts who deal with issues of reproductive aging and the possibility of iatrogenic acceleration is the education of professionals and the public regarding oncofertility issues, including ethical, legal, and social issues that develop in connection with this new direction in reproductive medicine (Woodruff, 2010).

The following text will highlight new possibilities for reproductive protection, their effectiveness, and long-term results related to reproductive function restoration after curative comprehensive treatment. The introduction addresses reproductive aging questions, the increasing incidence of reproductive age cancer, and the impact of fertility treatment among these patients. The mechanisms of damage to reproductive organs and tissues through the basic modalities of cancer treatment, including chemotherapy and radiotherapy, will be described. This book also clearly reviews the currently available methods for ovarian protection using modern techniques of assisted reproduction in combination with surgical and pharmacological treatment.

Subsequent chapters will introduce new techniques and methods of reproductive protection for reproductive age cancer patients that were gradually developed in the Department of Gynecology and Obstetrics of Masaryk University in Brno. The author details his results and experience with the application of these procedures in medical clinical practice. The final chapters discuss the social, ethical, and legal aspects of fertility loss after cancer treatment. This exciting medical field, which connects oncology and assisted reproduction (AR), requires interdisciplinary collaboration among an entire team of experts and strong individualization of the methods used to preserve patient reproductive health.

2 REPRODUCTIVE AGING

Martin Huser

During the past fifty years, there has been a clear trend toward the emancipation of women in society. Many women aim to postpone motherhood until they complete their education and build a career. Unfortunately, women pay a price for this choice. Fertility in women decreases exponentially with advancing age, especially after the age of thirty years. Although the nature of this phenomenon is not well known, we know based on numerous observations that reproductive aging is quite variable. For some women, fertility loss develops rather slowly, while other women suffer from unwanted infertility quite early.

The decline in fertility with age occurs in women of all races and has been recognized throughout human history. The age of 40 years is widely recognized as a limit for achieving pregnancy and a healthy birth, even though most women of this age continue to have regular menstrual cycles. Pregnancy potential is contingent upon the gradual decline in the number and quality of ovarian follicles. According to some experts, the optimum time for conception in women is between the ages of 18 and 30 years. After this period, an exponential decrease in the number of ovarian follicles takes place, as described in the 1990s by Faddy (Faddy et al., 1992).

The main cause of the swift decline in fertility with increasing age is not only a rapid decline in ovarian follicles but also an increased risk of early miscarriage. The miscarriage potential during the first trimester is approximately 10 percent at age 25 years and almost 50 percent at age 45 years. Miscarriage often occurs at a very early stage of fetal development and is often asymptomatic. This phenomenon is explained by the increase in the number of aneuploid embryos recorded within the context of AR after the introduction of preimplantation genetic diagnosis (PGD) methods. With advancing

age, both embryos and oocytes exhibit structural anomalies. At the age of 40 years, 80 percent of oocytes manifest cytoskeletal system anomalies during the first meiotic division. It is therefore obvious that the declining quality of oocytes with age is a major limiting factor for fertility in women.

However, even in old age, there are genetically normal oocytes and embryos. An aging endometrium and its quality can also be determining factors in declining fertility among women. Animal models demonstrate that reproductive results among proportionally older animals are poor, even when embryos carrying genetic material are transferred from juvenile gamete donors. Experience with human medicine, however, is quite different. Older patients who receive oocytes from healthy donors, mostly under the age of 30 years, have very good reproductive performance and a relatively small percentage of early miscarriages. This phenomenon can be partially explained by the fact that we supplement most donated oocyte recipients with supraphysiological ovarian steroid doses. Nevertheless, it appears that the endometrium plays an essential role in the decline in fertility with age.

On the issue of reproductive aging, we cannot completely underestimate the role of the aging hypothalamus and the secretion of ***gonadotropin-releasing hormone*** (GnRH). For example, the fact that menopausal women often experience problems despite a completely regular menstrual cycle cannot be clearly explained by the decline in the number of ovarian follicles. Hypothalamus aging and alterations in pulsatile GnRH production are also likely to affect a woman's reproductive outcomes. Notably, hypothalamic failure of GnRH pulsatility occurs later than the decline in fertility among women. This process probably results from the complete depletion of primordial follicles (PMFs).

The ovaries remain the key to reproductive aging among women. The aging of the ovaries depends mainly on the number of PMFs after birth and the speed of their atresia. In an effort to find answers to questions regarding reproductive aging in women, it is worth considering this key process in greater detail.

2.1 DETERMINED NUMBER OF OVARIAN FOLLICLES

During development of the fetus *in utero* during the first trimester, ovarian follicles form from stem cells, which migrate into the gonadal ridge and differentiate into oogonia. During this time, pregranulosa fetal cells migrate from the *mesonephros* into the ovary and create clusters around oogonia at the beginning of the second trimester. Then, the oogonia begin the first meiotic division, which ceases during the diplotene stage. Subsequently, the so-called primary oocytes form, which gradually surround themselves with granulosa cells and produce follicles. The number of oogonia begins to gradually decline from the 20th week of pregnancy, when they are estimated to number approximately seven million.

It is often erroneously reported that the ovary contains a static number of follicles. In contrast, this number continues to grow from the second half of pregnancy until birth. Oocytes gradually surround themselves with granulosa cells and form so-called ***PMFs***. An oocyte bounded by one layer of granulosa cells is defined as a ***primary follicle.*** The decrease in the number of primordial and primary follicles is likely to begin around childbirth, but these claims are supported by limited data. The number of PMFs during prenatal development steadily declines to approximately 30 percent during the 40th week of pregnancy. Conversely, the number of primary follicles increases and amounts to approximately 70 percent of follicles around the due date. It is therefore clear that at the end of the prenatal development of the fetus, most follicles are no longer in the primordial stage but in other stages of folliculogenesis (Faddy et al., 1992).

It remains a mystery exactly when and especially why some PMFs continue to develop from dormancy and begin to grow into the next stages of folliculogenesis. Therefore, it is very difficult on the basis of morphological characteristics to decide whether a follicle is in the dormant stage or whether it is already a growing follicle. For a long time, only PMFs were regarded as dormant follicles. Morphometric studies by Gougeon and colleagues showed that all types of follicles from the primary stage to folliculogenesis (including) are dormant follicles (Gougeon and Chainy, 1987). The transformation of follicles from the primordial stage through the intermediary stage to the

primary stage is likely to be a specific maturation process that can last for decades. The follicle-stimulating hormone (FSH) receptors on the cells of follicles occur early in the primary stage. It is possible that FSH, along with other endocrine and paracrine factors *(stem cell factor, growth differentiation factor 9, transforming growth factor beta, anti-Müllerian hormone and others),* plays a key role in initiating further follicle growth. If we fully understood these processes, we could influence the variable and personalized ratio of the number of follicles in a woman's body that are leaving the dormant folliculogenesis stage in a woman's body every day of her life.

The number of follicles in the ovary begins to decrease exponentially after a woman's birth. Anatomical studies have shown that a woman is born with 600 thousand to 700 thousand follicles. At the age of 20 years, approximately 80 thousand of these follicles survive. Their number progressively drops to approximately 3000 at the age of 45 years (Block, 1952). Mathematical models show that by the age of 37 years, the drop in the number of follicles is caused by the gradual maturation of follicles into the growing follicle stage. After reaching that age, there is also atresia of the dormant follicles, which never enter into the process of follicular growth. Therefore, the rate of follicle depletion significantly increases after reaching that age (Faddy and Gosden, 1995).

During the past ten years, we have also gained some interesting insights into the quality of the follicle pool in the ovary during a woman's life. Deteriorating quality of the follicles is explained by the accumulation of genetic defects on the basis of oxidative stress or toxins in the environment and food. These defects accumulate in oocytes in dormant follicles. The deterioration of the follicle quality is also sometimes explained by the decrease in blood circulation in the ovarian tissue that is related to a woman's age (Gaulden, 1992). Submicroscopic studies of oocytes in older women have shown changes in the morphology of mitochondria, which may have a direct effect on the reproductive potential of the entire oocyte (de Bruin et al., 2004). Mitochondrial DNA (mtDNA) is inherited along the maternal line and is far more prone to deletion mutations than its nuclear variant. Changes in mitochondrial DNA may therefore play an important role in the decline in oocyte quality during aging in

women. In addition, mitochondrial dysfunction plays an important role in the mechanism of cell apoptosis at the general biological level (Kitagawa et al., 1993).

The decrease in the number of follicles and deteriorating oocyte quality due to aging go hand in hand, and attempts to explain the correlations of these processes are still at the level of hypotheses. A decline in oocyte quality is likely to reduce the chance of conception and thus determine the definitive end of a woman's fertility period. Conversely, the decrease in the number of ovarian follicles causes changes in the menstrual cycle and thus determines the age of the onset of menopause (Sauer, 1998).

There are several molecular biological explanations for the decrease in the number and quality of follicles described above. Specific signals and metabolic processes in follicle cells have been described, which determine the folliculogenesis status of a given follicle and may lead to ***apoptosis*** or programmed cell death. Potential marker genes encoding these proteins come from the Bcl-*2* and *CASP40* gene families (Takai et al., 2003). For example, expression of the BAX gene family member *Bcl-2* in mouse models leads to programmed oocyte death (Rucker et al., 2000). Oocytes in mice deficient in this protein are not subject to programmed death during gametogenesis during fetal development or postnatally. Conversely, the sphingolipid ceramide was identified as an antagonist of the BAX protein. Mutant mice with sphingomyelinase enzyme deficiency, which results in ceramide protein activation, were also resistant to apoptosis, and these mice were born with far greater ovarian reserves than normal mice (Kolesnick and Krönke, 1998). In contrast, the metabolite ceramide sphingosine-1-phosphate (S1P) was identified in some types of ovarian cells as an inhibitor of apoptosis induced by ceramide (Olivera et al., 1999).

The observations of Johnson and Tilly (Johnson et al., 2004) in a mouse model demonstrated the formation of new PMFs from specific stem cells in the bone marrow *(stage-specific embryonic antigen 1, SSEA1)* and their implantation in the ovary and were quite significant, as they upset the theory that had been taught for decades about the predetermined lifetime decrease in the number of PMFs in the ovary of women (the so-called *numerus fixus* theory). This new

and revolutionary hypothesis would explain the sporadic cases of healthy birth among women with clearly demonstrated premature ovarian failure, often many years after the completion of anticancer chemotherapy. In 2006, the first case report in the world was published documenting spontaneous conception and healthy birth in a woman with defunct ovaries resulting from chemotherapy, several months after the autotransplantation of ovarian tissue into the forearm tissue (Oktay, 2006). In particular, the insertion of healthy, previously frozen, ovarian tissue into the body may activate oocyte production in the ovary contingent upon a still unknown mechanism.

2.2 FACTORS AFFECTING REPRODUCTIVE AGING IN WOMEN

Based on population studies, it is clear that the factors influencing the onset of menopause (pool depletion of ovarian follicles) may also accelerate the reproductive aging of women and increase the risk of premature ovarian failure. The length of the fertile period depends on the initial number of PMFs at a woman's birth. Their number may be genetically determined and is also certainly influenced by environmental factors, including tobacco smoke toxins, diet composition, and atmospheric pollution exposure during prenatal fetal development. Another factor influencing folliculogenesis may be a lack of nutrients during intrauterine growth retardation of the fetus (Goldenberg and Culhane, 2007).

Postnatal conditions may also accelerate ovarian follicle depletion and affect reproductive age. Iatrogenic factors, such as surgical removal of part of the ovarian tissue, the destruction of functional ovarian tissue by benign afflictions (e.g., *cysts, endometriosis, and tumors*), or the loss of ovarian follicles due to chemotherapy or radiation therapy for oncological diseases, play an important role.

Every gynecologist should be aware of how valuable a woman's ovarian reserve is before undertaking ovarian surgery. Even a simple laparoscopic extirpation of an ovarian cyst may significantly affect the size of the ovarian follicular pool. Additionally, follicular ovarian cysts, which are often detected by ultrasound in premeno-

pausal women (99 percent benign), should be primarily addressed in a conservative manner. If surgery is unavoidable, the surgeon should always preserve as much healthy ovarian tissue as possible. When cancer treatment with a high risk of permanent ovarian damage is necessary and the prognosis ***quod vitam*** is good, the oncologist should consider ovarian tissue acquisition and cryopreservation.

A variety of environmental factors that affect menopause onset have also been identified. For example, parity correlates positively with childbearing; in contrast, smoking accelerates the onset of menopause (Westhoff et al., 2000). Women of a higher socioeconomic status tend to experience menopause later.

Many population studies have also confirmed heredity as a factor that determines the age of menopause onset among mothers and their daughters. Data from national registries concerning monochorionic twins confirmed a significant influence of genetic factors on the onset of menopause. Promising studies are determining genes that affect and influence menopause onset. Several candidate genes have been found on the X chromosome or the q arm of chromosome 9 (Bruin et al., 2001). The deletion of these genes often correlates with the occurrence of premature ovarian failure. The discovery of these genes could serve to identify women at high risk for early onset of menopause and loss of reproductive capacity, enabling this group to become potential candidates for developing methods of reproduction protection.