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Huidong Zhang
Jie Yan
Editors

Environment and Female Reproductive Health

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Environment and Female Reproductive Health

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Foreword

Environment is the material basis for human survival and development. Reproductive health is the basic condition to support human survival and sustainable development in nature. The maintenance of human fertility is related to the population quality and future development of our world. The disorder of reproductive health is one of the primary causes of human fertility decline. The female reproductive process is much more complicated than male reproduction. Moreover, the female reproductive system is more fragile and more vulnerable to damage. In the long evolutionary process, human beings have formed the relationship between adaptation and dependence on the ecological environment, and the environment and reproductive health are in a dynamic balance.

Recently, increasing evidence shows that environmental harmful factors have become potential risk factors for female reproductive health, causing certain harms and also leading to many chronic diseases, even infertility and sterility. People pay more and more attention to the impact of environmental factors on reproductive health, especially on understanding the adverse factors before effective protection and medical treatment.

This book, edited by Huidong Zhang and Jie Yan, is timely and comprehensive. It introduced environmental harmful effects on female reproductive health, reviewed

the recent research progress, addressed the challenges, and also stimulated the development in the interdisciplinary area between environment and female reproductive health. In Part I, various environmental harmful factors were systematically and comprehensively introduced. In Part II, the female reproductive process and various reproductive diseases were introduced. In Part III, environmental effects on several important stages in the reproductive process were described. In Part IV, various reproductive diseases induced by environmental harmful factors were discussed. In Part V, how to preserve fertility is particularly important for female cancerous patients.

In summary, this book focuses on the environment and female reproductive health. It combined the basic research and clinical research and revealed the known toxicological mechanism in the process of female reproduction. This book is suitable for many readers. It not only has authoritative scientific guidance value for many medical professionals but also has authoritative health science popularization significance for people of childbearing age. It is suitable for basic researchers and clinicians in reproductive medicine, tumor medicine, etc. The editors and authors deserve to be congratulated for their excellent work.

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About the Editors

Huidong Zhang is Professor of Sichuan University. He has obtained various awards such as Thousands of Youth Talent Plan in China, Scientific Foundation for Outstanding Youth Scientist in China, the First Prize of Liaoning Science and Technology Award, Outstanding Youth Science and Technology Award of Chinese Society of Toxicology, Leading Talents of Sichuan Health and Family Planning Commission, Sichuan Thousand Talents Plan, Youth Science and Technology Talent Award in Sichuan University, Academic and Technical Leader of Sichuan Provincial Health Committee, etc. His group focuses on research in the environment and female reproductive health. As the first or corresponding author, he has published 49 papers in various publications, such as *PNAS, NAR, JBC, MRR,* and *EP*, including six invited papers for special topics, covers, or feature articles. These papers have been cited 1337 times, including by *Science, Nat Commun, PNAS, NAR,* etc. He has been selected as the member or standing member of 15 Chinese Academic Committees, including toxicology, reproductive health, and enzymology areas. His research interests are to explore the roles and functions of noncoding RNAs in the BPDE-induced dysfunctions of trophoblast cells and female abortion.

Jie Yan is Associated Professor of Peking University Third Hospital. Her research group focuses on basic and clinical translation research on human fertility maintenance and preservation. She has clarified the important roles of *in vivo* microenvironment in human follicular development, revealed the regulation mechanism of microenvironment factors for human follicle development *in vitro*, established OP-IVM "egg bank" that has significantly improved pregnancy outcome, analyzed the bionic ice control mechanism of germ cell cryopreservation, and invented the novel cryopreservation medium with a natural amino acid as the core component that has already been translated. She has published 69 papers in *Mol Cell* or other international or national authoritative academic journals, which have been totally cited 1733 times (H-index 20). She has won seven national authorized patents and the First Prize of Chinese Medical Science and Technology Award.

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Part I
Environmental Harmful Factors

Chapter 1
Introduction to Environmental Harmful Factors

Jiarong Guo, Peng Tian, Zhongyan Xu, and Huidong Zhang

Abstract In this Chapter, we systematically and comprehensively described various environmental harmful factors. They were classified into four aspects: physical factors, chemical factors, biological factors, and physiological and psychological stress factors. Their classification, modes of presence, toxicity and carcinogenicity, routes of exposure to human and toxic effects on the female reproductive health were introduced. It is expected that the exposure routes could be controlled and eliminated, and the pathogenic mechanism of environmental harmful factors should be investigated and explained to protect female reproductive health.

Keywords Environmental harmful factors · Physical and chemical factors · Biological factors · Physiological and psychological stress factors · Female reproductive health

1.1 Introduction

Various environmental factors are closely related to human health. When some chemicals are adsorbed by plants, they enter food chain and would cause primary threat to human health [1]. The particulate matter (PM), which comes from metals, organic carbon, vehicles, and biomass burning emissions, could lead to oxidative stress and impact human health [2]. Various viruses, such as COVID-19, also threaten human health. All these factors those may have adverse effects are considered as environmental harmful factors. In general, environmental harmful factors include physical factors, chemical factors, and biological factors. However, the term "exposome" includes both external and internal factors as well as human's response to these factors [3]. External environmental factors determine external exposure

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dose; and the internal exposure is dependent on the exposure pathway and the response of human. Finally, environmental factors may cause differential effects on organism due to the additional psychological influence. For example, air pollution is likely to cause greater illness in those with heavier exposure and greater susceptibility [4]. Hence, in this Chapter, we will introduce the environmental harmful factors from the following four aspects: physical factors, chemical factors, biological factors, and physiological and psychological stress factors.

1.2 Physical Harmful Factors

Physical factors mainly include noise, vibration, electromagnetic radiation, and meteorological conditions. These physical factors usually have their definite sources. Except for laser, other factors could all be found in nature. Some of the factors in an appropriate dose would be necessary and non-harmful for human. However, extra strong dose or radiation in other wavelength range would be harmful for human.

1.2.1 Noise

In physics, noise refers to the sound with irregular frequency or intensity. In hygiene, the annoying or unnecessary sounds are considered as noise. According to its source, noise can be divided into natural noise and unnatural noise. Noise mainly affects the auditory system. Short-term exposure to noisy environment may lead to auditory adaptation or fatigue, which belongs to temporary threshold shift and can be recovered after a certain period of time. However, if persons remain in a noisy environment for a prolonged period of time, it will lead to permanent threshold shift, including hearing loss and deafness [5, 6]. Noise-induced hearing loss is more common in work, which could be considered as a kind of occupational disease [7]. Noise can also affect the nervous system, circulatory system, digestive system, and psychology. High levels of traffic noise are not only associated with depression and anxiety disorders, but also related to cardiovascular and metabolic disorders [8–10]. Furthermore, noises are also associated with female reproductive health. A study in China found a link between noise and menstrual disorders in female health workers and nurses [11]. This noise may ultimately affect women's reproductive health. Another experiments show that exposure to modest to high levels of noise significantly decreases the reproductive efficiency of mice by reducing the quantity of pups born and increasing the quantity of stillborn pups [12]. Therefore, it is necessary to set noise standards for environmental and industrial enterprises, to control noise sources and noise propagation, and to perform individual protection and health monitoring against noise.

1.2.2 Vibration

Vibration refers to the repeated motion of a particle or an object in a straight line or arc around an equilibrium position under the action of external forces. It can be divided into local vibration and whole-body vibration. Local vibration is caused by hand contact with vibrating tools or machinery, and can be transmitted through the arm to the whole body. Local vibration will cause vibration damage to fingers, hands, and arms, even induce hand-arm vibration syndrome [13]. Whole-body vibration refers to the vibration that is transmitted to the whole body through the lower limbs or trunk. The situations may cause whole-body vibration, including taking a vehicle, operating agricultural machinery such as tractors, and locating on the platforms such as drilling platforms. Whole-body vibration can increase heart rate, cause dizziness, nausea, and other symptoms, even lead to motion sickness. Experiments on tall buildings confirmed that vibration can cause motion sickness and sopite syndrome (sleepiness) [14, 15]. And long-term whole-body vibration exposure can probably contribute to the disorders of female reproductive organs and disturbances of pregnancy (abortions, stillbirths) [16]. Animal studies further confirmed that vibration is indeed relevant with reproductive health [17]. Eliminating the source of vibration is the fundamental way to prevent vibration hazard.

1.2.3 Ionizing Radiation and Non-ionizing Radiation

Ionizing radiation could cause substances to be ionized. According to the source, it can be divided into natural radiation and artificial radiation. Natural radiation includes cosmic rays and radioactive elements in crustal rocks. Artificial radiation includes x-rays, gamma rays, protons, and so on, which are produced by ray generators. Ionizing radiation exists in nuclear industrial systems, in the production and processing of radioactive elements, production and use of ray generators, and medical processes. Excessive ionizing radiation will affect human health. Radiotherapy used to treat brain tumors may damage the central nervous system, then affect the peripheral immune system [18]. A cohort study has suggested that the occurrence rate of cancer is increased linearly with the increase of radiation exposure [19]. For the health of the fetus, ionizing radiation must be kept away during pregnancy. Gadolinium MRI exposure at any stage of gestation may increase maternal and fetal health risks [20]. Another study shows that occupational exposures of ionizing radiation are positively correlated with spontaneous abortion in female veterinarians [21]. Thus, it is better to avoid radiation by shielding and isolation from the source.

Non-ionizing radiation cannot cause ionization of biological tissues. It belongs to a specific range in the electromagnetic radiation spectra, and mainly includes radiofrequency (RF) radiation, infrared, visible light, ultraviolet, and laser. Since 2011, International Agency for Research on Cancer (IARC) at WHO identified

radiofrequency radiation in the 30–300 GHz range as a "possible" human Group 2B carcinogen [22]. Humans are inevitably exposed in the process of industrial heating, communication, radio positioning, and radar navigation, which can affect the nervous system, reproductive system, blood, and eyes. Animal studies have shown that radiofrequency electromagnetic radiation can lead to learning and memory impairment, decrease the activity of brain antioxidant enzymes, increase the concentration of corticosterone and lipid peroxidation, and cause histopathological aberrations in the hippocampal tissues [23]. Rat assays showed that long-term exposure to radiofrequency electromagnetic radiation from 4G smartphone will diminish male fertility through directly interfering the Spock3-MMP2-BTB pathway in rats [24]. Infrared radiation is generally found in sunlight and strong luminaries (tungsten, neon, infrared searchlight), and it is also present in the process of steel making and welding. Sun, ultraviolet (UV) lamp, welding, smelting, and other processes produce UV. Both infrared and UV radiation can hurt skin and eyes. Solar radiation contains infrared, ultraviolet, and visible light, and they damage human skin [25]. The development of possibly age-related macular degeneration, pterygium, and cataracts may be associated with UV radiation exposure [26]. Therefore, staying away from radiation sources and taking personal precautions are very important to protect human from radiation.

1.2.4 Abnormal Meteorological Conditions

A suitable external environment is important to maintain human health. High temperature directly affects the regulation of body temperature and metabolism and also influences other systems. Heat stroke is a typical disease caused by high temperature. Extreme high temperature appears to increase the incidences of cardiovascular and respiratory disorders [27]. Animal study confirmed that heat stress had a detrimental effect on female fertility in cows [28]. Kawasaki disease (KD) incidence is significantly affected by temperature. It has been found that KD is negatively associated with the temperature from February to May [29]. In addition, low temperature caused by cold environment, high pressure caused by diving, and other low-pressure environments also have impacts on human health. Thus, climate change threatens our living environment and also affects human health.

1.3 Environmental Harmful Chemicals

Many environmental chemical substances are environmental endocrine disruptors (EEDs) or persistent organic pollutants (POPs). The normal functions of the endocrine system of humans can be altered by EEDs. Human are exposed to EEDs due to their occupation, diet, and living environment [30]. POPs are accumulated in the food chain. POPs have the characteristics of long-distance transportation,

persistence, bioaccumulation, and high toxicity [31]. Polycyclic aromatic hydrocarbons (PAHs) and bisphenol A are typical EEDs and POPs.

1.3.1 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are organic substances consisting more than two fused aromatic rings. The US EPA and the European Food Safety Authority (EFSA) list PAHs in the priority-pollutant lists. PAHs can act as EEDs and cause endocrine disruption on general population. PAHs are classified as known, possibly, or probably carcinogenic to humans (Group 1, 2 A, or 2B) [32].

PAHs are highly mobile and widely distributed in our daily environment. Both natural and anthropogenic processes generate airborne PAHs. The primary airborne PAHs originate from coal/biomass burning [33]. In atmosphere, low-weight PAHs ($\leq$202 g/mol, generally with 2~4 rings) tend to present in vapor phase [34]. The average concentrations of PAHs are higher in fog than in rain [35]. Furthermore, the low-weight PAHs can react with other air pollutants and form toxic compounds [34]. High-weight PAHs ($\geq$252 g/mol, generally with more than four rings) are hardly vaporized and tend to adhere to particulates. Particulate PAHs account for 5%–39.8% of total PAHs [36]. Particulate PAHs mainly deposited to oily substances such as soil [37].

In soil, benzo[b]fluoranthene, pyrene, and fluoranthene are main types of PAHs [38]. In UK, 90% of total PAHs are stored in soil [39], suggesting that soil is the largest reservoir for environmental PAHs. The distribution of PAHs in topsoil is not uniform. In China, the concentration of PAHs in urban topsoil (0.030–23.300 μg/g) is higher than that in rural areas (0.0037–6.250 μg/g) [40]. On the whole, the highest PAHs concentration (1.467 μg/g) was found in Northeast China and the lowest PAHs concentration (0.209 μg/g) was found in West China [41].

In the aquatic environment, PAHs range from 0.03 ng/L to 8,310,000 ng/L in atmosphere and waste water [42]. PAHs are preferentially absorbed onto organic matter and might be re-released into liquid phase [43]. In general, the solubility of PAHs diminishes as the molecular weight increases. High-weight PAHs such as benzo[a]pyrene and chrysene could attach to particulate matter. Thus, the contaminated sediment represents a permanent source of PAHs pollution.

The main ways for the general population to be exposed to PAHs are eating grilled food or breathing air from an open fireplace or smoke. Asian children in polluted areas were exposed to high concentrations of particulate PAHs [44]. Workers in automobile workshop, iron foundries, and aluminum plant have a high risk of exposing PAHs [45].

Many studies have shown that PAHs have genotoxicity, carcinogenicity, mutagenicity, and developmental toxicity. Exposure to PAHs is associated with dysfunction of many organs and diabetes and reproductive disorders [46]. PAHs seem to affect reproductive regulators such as follicle stimulating hormone. PAHs disrupted placental physiology, trophoblast migration, and uterus and cervix [47]. PAHs can

also potentially cause cell damage and induce cytotoxicity and pro-inflammatory responses [48]. Monooxygenase-catalyzed PAH-ligand/AhR activation could add an electrophilic group to PAHs, and generate genotoxic metabolites. Genotoxic metabolites may form adducts with DNA and finally induce DNA damage [49]. However, the toxicological effects of PAHs in most contaminated sites may be underestimated. Nitrogen, sulfur and oxygen (N/S/O)-heterocyclic PAHs, nitrated PAHs (N-PAHs), and oxygenated PAHs (oxy-PAHs) have more potential toxicity but were generally not taken into account [50].

In summary, epidemiological studies have shown that PAHs are harmful to human health. Cellular and molecular biological studies have clarified how PAHs lead to dysfunctions of cells. However, it is difficult to assess the attribution of PAHs to various diseases occurrence and development [51]. Network biology confirmed that PAHs play crucial roles in the occurrence of diseases [52]. With the evolution of new technologies, the disease mechanism induced by PAHs will be clarified.

1.3.2 Bisphenol A

The plastics industry use bisphenol A (2,2-bis [4-hydroxyphenyl] propane, BPA) as monomer in the production of polymer materials (mainly epoxy resins and polycarbonate plastics). BPA is also widely used as raw material for daily necessities [53]. BPA principally through manufacturing and wastewater treatment processes is released into environment. BPA's water phase solubility at 25 °C is 300 mg L^{-1}. At ambient temperature BPA is not volatile, but it can be absorbed into soil, sediments, and solid matrix to form binding residues. In an aerobic environment, BPA could be metabolized by different taxa of algae, fungi, bacteria, and even higher plants. In the absence of oxygen, abiotic processes mediate the conversion and mineralization of BPA [54].

Humans can be exposed to BPA through food, beverages, wastewater, air, dust, and soil [55]. Diet is the main exposure pathway for BPA and its analogues in general population [56]. Breast milk is a source of BPA exposure for infants. BPA enters milk in the milk production process and dairy products, which poses a threat to human health [57].

EEDs affect the reproductive health of male and female [58]. BPA, a poison of ovary, uterus, and prostate, may induce hypomethylation in women and young girls [59]. In placenta, BPA increases inflammation and oxidative stress and decreases cell viability [60]. BPA shows negative effects on implantation and the occurrence of polycystic ovary syndrome. BPA impairs sperm quality, but it is not clear whether it leads to poor reproductive outcomes and sexual dysfunction in men [61]. Moreover, prenatal exposure to BPA might lead to neurobehavioral disorders and adverse behavioral outcomes in children [62].

Exposure to BPA is more likely to suffer from diabetes, general/abdominal obesity, and hypertension [63]. As a metabolic disruptor, BPA causes abnormal epigenetic disorders. BPA impacts DNA methylation, histone demethylation, and

deacetylation of glucose homeostasis-related genes. BPA also impacts glucose and lipid balance, and impairs insulin signal transduction [64]. Finally, BPA induces dysfunction of energy balance control system, leading to obesity and type II diabetes [65].

Exposure to BPA can lead to bone loss. BPA reduces the level of blood calcium, inhibits the secretion of calcitonin, and blocks bone metabolism [66]. Early BPA exposure leads to childhood wheeze/asthma [67], breast cancer, and prostate cancer [68].

Long non-coding RNAs (lncRNAs) could be biomarkers or key regulators of toxicological responses. The expressed profile of lncRNAs is correlated with the toxicity of chemicals [69]. For example, BPA causes hypomethylation of DNA and reduces the expression of Igf2 and H19, one possible mechanism in which BPA induces epigenetic regulation on male fertility [70]. However, much more studies should be further performed to study the roles of lncRNAs as biomarkers or intervention targets in biological regulation.

In addition, other EEDs also threaten human health in all aspects of our lives. For example, neurotoxic pesticides including DDT and chlorpyrifos are widely used in agriculture. Heavy metals such as lead are widely applied in refining, jewelry, mining, batteries, and children's products. Lead has neurological effects and threat health in various ways [71]. Epidemiological evidences should be correlated with the molecular mechanisms obtained in in vitro experiments.

1.4 Biological Harmful Factors

The exposure of biological harmful factors links to poor outcomes like preterm delivery and stillbirth [72]. These factors can be inhaled, ingested, and contacted via ocular and dermal, then threat female health [73]. For instance, it is generally believed that abortion is related to sexually transmitted infections such as *Treponema pallidum*, *Neisseria gonorrhoeae,* and *Chlamydia trachomatis*. Sexually transmitted viruses including human papillomavirus (HPV), human herpes virus (HSV), human cytomegalovirus (HCMV), adeno-associated viruses, and human immunodeficiency virus (HIV) may also link to reproductive alternations [74]. Hence, we review researches that have explored the association between abortion and infection. The pathogens and their relation with abortion were summarized in Table 1.1.

1.4.1 Bacterial Vaginosis

Lactobacillus species bacteria account for a large proportion of the normal genital tract flora in healthy women. Bacterial vaginosis (BV) means other virulent organisms that can replace *lactobacilli* as the main organisms in the vagina. These virulent

Table 1.1 Summary of pathogens and their relation with abortion

	Bacteria	Viruses	Protozoa
Related with abortion	• Bacterial vaginosis (including *Mycoplasma hominis and Ureaplasma urealyticum*) • *Brucellosis* • *Syphilis* • *Coxiella burnetii* • *Mycoplasma genitalium* • *Chlamydia trachomatis* • *Neisseria gonorrhoeae* • *Clostridium sordellii* (*C. perfringens*) • *Listeria monocytogenes*	• Cytomegalovirus • Dengue fever (*Flavivirus)* • HIV • Rubella • Adeno-associated virus • Bocavirus • Hepatitis C • Human papillomavirus • Herpes simplex virus 1 and 2 • Parvovirus B19 • Polyomavirus BK • Hepatitis B	• Malaria (*Plasmodium*) • *Toxoplasma gondii* • *Trypanosoma cruzi* • Schistosoma (*Schistosoma haematobium* and *Schistosoma mansoni*)

organisms have been related with miscarriage and premature delivery, including *Mycoplasma hominis,* group B streptococci, *Gardnerella vaginalis,* group B streptococci, *Staphylococcus aureus,* or *Ureaplasma urealyticum* [75]. Further cohort study includes 759 Belgian pregnant women, 8.4% participants developed BV and did not received treatment. It was found that BV was positively associated with abortion [76].

1.4.2 Virus Infection

1.4.2.1 Human Papillomavirus (HPV) Infection

HPV belongs to sexually transmitted infection. HPV infection is associated with infertility of women. In spontaneous abortion samples, the incidence of HPV infection is three times higher than that of elective abortion [77]. Cervical HPV-infected patients have fewer pregnancies [78]. HPV infection of trophoblasts corrupts the embryo's health by invading the uterine wall. HPV16 also results in trophoblast cell apoptosis, which causes placenta dysfunctions and reduces embryo ability, leading to miscarriage [79].

1.4.2.2 Human Immunodeficiency Virus (HIV) Infection

Negative pregnancy outcomes are related with maternal HIV infection. Pregnant HIV-infected women have a higher risk to spontaneous abortions and stillbirth, and their infants are more frequently born with a low birth weight [80]. HIV infection leads to chronic inflammation with the increased activated innate cells and increases granulocyte-macrophage colony stimulating factor (GM-CSF) [81, 82]. This proinflammatory shift has a link with spontaneous abortion and preterm birth.

1.4.2.3 Other Viruses Infection

In addition, other viral infections are also associated with abortion in women. For instance, syphilis is a bacterial infection, that can be transmitted sexually or via contact with blood of an infected person. Syphilis can cause stillbirth, abortion, and congenital transmission [83]. Cytomegalovirus infection leads to placental dysfunctions and spontaneous abortion [84]. Studies found that Rift Valley fever virus and miscarriage have a link in Sudanese women [85]. Influenza virus may increase the risk of premature delivery in pregnant women [86]. H1N1 influenza virus infection can cause the dysregulation of inflammatory responses, result in pre-term labor, impairment of fetal growth, and fetal mortality [87].

1.4.3 Toxoplasma Infection

The prevalence of toxoplasmosis varies around the world [88]. Alvarado et al. conducted a survey of 326 women with an abortion history, founded that 6.7% of them had been exposed to T. gondii [89]. For IgG against T. gondii, 55% of 100 women were seropositive [90]. The data suggests that there is a higher occurrence of toxoplasma infection if women with abortion.

1.5 Physiological and Psychological Factors

Female health is dependent on physical, mental, and social well-being. Social and economic status, social support, quality of marriage, childbearing age, psychological stress, and unhealthy lifestyle all affect female fertility health [73]. Besides, subfertility, amenorrhea, and poor endometrial development may relate to excessive exercise, calorie restriction, and over diet [91].

1.5.1 Adverse Childhood Experiences

Early life experiences are very important for health throughout the life course. Individual adults who have adverse childhood experiences (ACEs) tend to have more mental and physical health problems and premature mortality [92]. ACEs also potentially affect maternal health outcomes during pregnancy. It is vulnerable to prenatal, perinatal, and postnatal psychosocial health if mothers have adverse childhood experiences. ACEs are associated with mental health risks during pregnancy. These problems include higher depressive symptoms, anxiety, and suicidality [93]. ACEs-related mental and behavioral health conditions are also associated with poorer child health [94]. Women who have ACEs are more susceptible to disease development. ACEs are important hazards for poorer maternal mental and behavioral health during pregnancy [95].

1.5.2 Age

Several other factors are associated with miscarriage. Increasing woman ages reduces conception rates. If both parents are 35 years age or older, the risk of adverse pregnancy outcomes increases. The risk is increased up to 50% if the mother is 42 years old [96]. The developing embryo metabolism might be different, dependent on the age of the mother [97]. Oocytes took from elder women may have higher risk of miscarriage and epigenetic modification [98].

1.5.3 Unhealthy Lifestyle

Infertility is related with body mass index (BMI). It has a similar risk of infertility for overweight (BMI 25–29.9 kg/m^2) and underweight (BMI < 19 kg/m^2). Overweight women have less possibility to ovulate and conceive naturally, and have a higher risk of miscarriage [99]. Obesity results in mitochondrial dysfunction in oocyte, increased granulosa apoptosis, and slower growth and delayed maturation of the oocyte [100].

Regular exercise could prevent diabetes, gestational hypertensive disorders, and fetal growth impairments [101]. However, overexercise could result in negative consequences of conception. Exercise 4 h or more per week for women during the first IVF cycle had a 40% reduction in live births. The risk of implantation failure is tripled, and the risk of miscarriage also became higher.

1.5.4 Double Stress of Work and Life

Psychological stress generally influences female fertility. Positive emotions are associated with an increased chance of having a living baby. When anxiety levels get higher, the chances of stillbirth will increase [102]. Women who worked 16–32 h a week experienced a shorter time to conception than those who worked more than 32 h a week [103]. Long-term stress in women can also lead to changes in the development of the immune systems, endocrine, and immune systems. This results in emotional, social, and cognitive functions impaired, as well as the allostatic load (i.e., chronic physiological damage) increased. Mechanically, stress may affect follicular stimulating hormone and pituitary luteinizing hormone (LH) pulsatility [104]. The reduction in luteal phase progesterone, LH, and serum estradiol concentrations may have associations with higher daily stress levels [105]. Reducing stress levels by therapeutic interventions may help women to return to normal ovulation.

1.5.5 High Altitude or Chronic Hypoxia

It is a great burden to pregnancy at high altitude for both fetuses and mothers. High altitude exposure and chronic hypoxia residence increase the occurrence of neonatal morbidity and pregnancy complications. These adverse outcomes include neurobehavioral disorders in neonates, aberrant organ development, and intrauterine growth restriction [106]. Hypoxia can delay the menarche time of women in high altitude area [107, 108]. When people living at sea-level visited higher altitudes, their menstrual cycle was changed. When the high altitude native population migrates to lower altitude, the menarcheal ages did not change, because their physiology has already adapted to high altitude condition. Besides, hypoxia induces immediate release of catecholamines, including cortisol and gonadotrophins [109]. These changes ultimately delay the follicular maturation, prevent or delay the ovulation, and affect implantation and pregnancy. Hypoxia also leads to the generation of ROS [110]. Excessive ROS has deleterious effects on female reproductive system. Chronic hypoxia reduces NO-dependent vasodilation in myometrial arteries, thus raising uterine vascular resistance, lowering uterine artery blood flow, and leading to hypoxia-related fetal growth restriction [111].

1.5.6 Occupational Exposure

Occupational exposure to reproductive system diseases has important influence on workers. There are many opportunities to be exposed to chemicals and other materials that may be harmful to the reproductive health of men and women. Pesticides and heavy metals on human reproduction have many negative side effects.

Pesticides reduce sperm concentrations by as much as 60% and result in oligozoospermia [112]. Occupational exposure to organic solvents decreases implantation rates [113]. Occupational exposure leads to teratospermia and asthenospermia [114]. Painters may be exposed to lead-based paints; lead may be contacted by crafters who making stained, ceramics, and jewelry; and gardeners may be exposed to pesticides. These contacts possibly reduce fertility. In another survey of 14,614 female workers in China, the female workers who work in the electronics, railway, and medicine and health industries have serious reproductive health problems [115].

In summary, living factors and environment may have influence on fertility, but then can be modified. These factors include age, psychological stress, nutrition, alcohol consumption, cigarette smoking, occupational exposures, exercise, environmental and other behaviors. Since these factors are ultimately under our control, we can subjectively choose our living environment and lifestyle to avoid them.

1.6 Conclusion

In conclusion, physical, chemical, and biological factors act on our bodies in specific and objective ways. Body's responses to subjective factors determine the internal exposure. Combination with psychological responses, these objective and subjective factors decide the final effects on organism. It is expected that the exposure routes could be controlled or eliminated, the pathogenic mechanism of environmental harmful factors could be investigated and explained to protect human reproductive health.

References

1. Burger A, Lichtscheidl I. Strontium in the environment: review about reactions of plants towards stable and radioactive strontium isotopes. Sci Total Environ. 2019;653:1458–512.
2. Bates JT, Fang T. Review of acellular assays of ambient particulate matter oxidative potential: methods and relationships with composition. Sour Health Eff. 2019;53(8):4003–19.
3. Krutmann J, et al. The skin aging exposome. J Dermatol Sci. 2017;85(3):152–61.
4. Schraufnagel DE, et al. Air pollution and noncommunicable diseases: a review by the forum of international respiratory societies' environmental committee, part 2: air pollution and organ systems. Chest. 2019;155(2):417–26.
5. Ding T, Yan A, Liu K. What is noise-induced hearing loss? Br J Hosp Med (Lond). 2019;80 (9):525–9.
6. Graydon K, et al. Global burden of hearing impairment and ear disease. J Laryngol Otol. 2019;133(1):18–25.
7. Lie A, et al. Occupational noise exposure and hearing: a systematic review. Int Arch Occup Environ Health. 2016;89(3):351–72.

8. Generaal E, et al. Not urbanization level but socioeconomic, physical and social neighbourhood characteristics are associated with presence and severity of depressive and anxiety disorders. Psychol Med. 2019;49(1):149–61.
9. Kempen EV, et al. WHO environmental noise guidelines for the European region: a systematic review on environmental noise and cardiovascular and metabolic effects: a summary. Int J Environ Res Public Health. 2018;15(2):379.
10. Eze IC, et al. Long-term exposure to transportation noise and air pollution in relation to incident diabetes in the SAPALDIA study. Int J Epidemiol. 2017;46(4):1115–25.
11. Jiang Z, et al. Menstrual disorders and occupational exposures among female nurses: a nationwide cross-sectional study. Int J Nurs Stud. 2019;95:49–55.
12. Rasmussen S, et al. Construction noise decreases reproductive efficiency in mice. J Am Assoc Lab Anim Sci. 2009;48(4):363–70.
13. Lai S-K, et al. A human-based study of hand–arm vibration exposure limits for construction workers. J Vib Eng Technol. 2019;7(4):379–88.
14. Lamb S, Kwok KCS. Sopite syndrome in wind-excited buildings: productivity and wellbeing impacts. Build Res Inf. 2017;45(3–4):347–58.
15. Lamb S, Kwok KCS. The effects of motion sickness and sopite syndrome on office workers in an 18-month field study of tall buildings. J Wind Eng Ind Aerodyn. 2019;186:105–22.
16. Seidel H. Selected health risks caused by long-term, whole-body vibration. Am J Ind Med. 1993;23(4):589–604.
17. Atanasov NA, et al. Characterization of train-induced vibration and its effect on fecal corticosterone metabolites in mice. J Am Assoc Lab Anim Sci. 2015;54(6):737–44.
18. Zhang P, et al. The effect of gamma-ray-induced central nervous system injury on peripheral immune response: an in vitro and in vivo study. Radiat Res. 2019;192(4):440–50.
19. Richardson DB, et al. Risk of cancer from occupational exposure to ionising radiation: retrospective cohort study of workers in France, the United Kingdom, and the United States (INWORKS). BMJ. 2015;351:h5359.
20. Ray JG, et al. Association between MRI exposure during pregnancy and fetal and childhood outcomes. JAMA. 2016;316(9):952–61.
21. Shirangi A, Fritschi L, Holman CD. Maternal occupational exposures and risk of spontaneous abortion in veterinary practice. Occup Environ Med. 2008;65(11):719–25.
22. Koppel T, et al. Radiofrequency radiation from nearby mobile phone base stations-a case comparison of one low and one high exposure apartment. Oncol Lett. 2019;18(5):5383–91.
23. Akefe IO, et al. C-glycosyl flavonoid orientin alleviates learning and memory impairment by radiofrequency electromagnetic radiation in mice via improving antioxidant defence mechanism. Asian Pac J Trop Biomed. 2019;9(12):518.
24. Yu G, et al. Long-term exposure to 4G smartphone radiofrequency electromagnetic radiation diminished male reproductive potential by directly disrupting Spock3-MMP2-BTB axis in the testes of adult rats. Sci Total Environ. 2020;698:133860.
25. Hudson L, et al. Individual and combined effects of the infrared, visible, and ultraviolet light components of solar radiation on damage biomarkers in human skin cells. FASEB J. 2020;34(3):3874–83.
26. Lucas RM, Yazar S. Human health in relation to exposure to solar ultraviolet radiation under changing stratospheric ozone and climate. Photochem Photobiol Sci. 2019;18(3):641–80.
27. Lin S, et al. Extreme high temperatures and hospital admissions for respiratory and cardiovascular diseases. Epidemiology. 2009;20(5):738–46.
28. Gernand E, König S, Kipp C. Influence of on-farm measurements for heat stress indicators on dairy cow productivity, female fertility, and health. J Dairy Sci. 2019;102(7):6660–71.
29. Abrams JY, et al. Increased Kawasaki disease incidence associated with higher precipitation and lower temperatures, Japan, 1991-2004. Pediatr Infect Dis J. 2018;37(6):526–30.
30. Sargis RM, Simmons RA. Environmental neglect: endocrine disruptors as underappreciated but potentially modifiable diabetes risk factors. Diabetologia. 2019;62(10):1811–22.

31. Lohmann R, et al. Global fate of POPs: current and future research directions. Environ Pollut. 2007;150(1):150–65.
32. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum. 2010;92:1–853.
33. Yi ZG, et al. Characteristics of PAHs in the atmosphere in winter and summer in the urban and suburban of Fuzhou. Huan Jing Ke Xue. 2013;34(4):1252–7.
34. Park JS, Wade TL, Sweet S. Atmospheric distribution of polycyclic aromatic hydrocarbons and deposition to Galveston Bay, Texas, USA. Atmos Environ. 2001;35(19):3241–9.
35. Li X, et al. Characterization of polycyclic aromatic hydrocarbons in fog-rain events. J Environ Monit. 2011;13(11):2988–93.
36. Gao B, et al. Source apportionment of atmospheric PAHs and their toxicity using PMF: impact of gas/particle partitioning. Atmos Environ. 2015;103:114–20.
37. Kim KH, et al. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Int. 2013;60:71–80.
38. Jiang Y, et al. Contamination, source identification, and risk assessment of polycyclic aromatic hydrocarbons in agricultural soil of Shanghai, China. Environ Monit Assess. 2011;183 (1–4):139–50.
39. Wild SR, Jones KC. Polynuclear aromatic hydrocarbons in the United Kingdom environment: a preliminary source inventory and budget. Environ Pollut. 1995;88(1):91–108.
40. Ma WL, et al. Polycyclic aromatic hydrocarbons in Chinese surface soil: occurrence and distribution. Environ Sci Pollut Res Int. 2015;22(6):4190–200.
41. Zhang P, Chen Y. Polycyclic aromatic hydrocarbons contamination in surface soil of China: a review. Sci Total Environ. 2017;605-606:1011–20.
42. Mojiri A, et al. Comprehensive review of polycyclic aromatic hydrocarbons in water sources, their effects and treatments. Sci Total Environ. 2019;696:133971.
43. Patrolecco L, et al. Occurrence of priority hazardous PAHs in water, suspended particulate matter, sediment and common eels (Anguilla anguilla) in the urban stretch of the River Tiber (Italy). Chemosphere. 2010;81(11):1386–92.
44. Oliveira M, et al. Children environmental exposure to particulate matter and polycyclic aromatic hydrocarbons and biomonitoring in school environments: a review on indoor and outdoor exposure levels, major sources and health impacts. Environ Int. 2019;124:180–204.
45. Ali N, et al. Polycyclic aromatic hydrocarbons (PAHs) in the settled dust of automobile workshops, health and carcinogenic risk evaluation. Sci Total Environ. 2017;601-602:478–84.
46. Idowu O, et al. Beyond the obvious: environmental health implications of polar polycyclic aromatic hydrocarbons. Environ Int. 2019;123:543–57.
47. Bolden AL, et al. Polycyclic aromatic hydrocarbons and female reproductive health: a scoping review. Reprod Toxicol. 2017;73:61–74.
48. Niu X, et al. Atmospheric levels and cytotoxicity of polycyclic aromatic hydrocarbons and oxygenated-PAHs in PM(2.5) in the Beijing-Tianjin-Hebei region. Environ Pollut. 2017;231 (Pt 1):1075–84.
49. Baird WM, Hooven LA, Mahadevan B. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ Mol Mutagen. 2005;45(2–3):106–14.
50. Andersson JT, Achten C. Time to say goodbye to the 16 EPA PAHs? Toward an up-to-date use of PACs for environmental purposes. Polycycl Aromat Compd. 2015;35(2–4):330–54.
51. Briggs D. Environmental pollution and the global burden of disease. Br Med Bull. 2003;68:1–24.
52. Iida M, Takemoto K. A network biology-based approach to evaluating the effect of environmental contaminants on human interactome and diseases. Ecotoxicol Environ Saf. 2018;160:316–27.
53. Staples CA, et al. A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere. 1998;36(10):2149–73.

54. Im J, Löffler FE. Fate of bisphenol A in terrestrial and aquatic environments. Environ Sci Technol. 2016;50(16):8403–16.
55. Valentino R, et al. Bisphenol A environmental exposure and the detrimental effects on human metabolic health: is it necessary to revise the risk assessment in vulnerable population? J Endocrinol Investig. 2016;39(3):259–63.
56. Russo G, et al. Occurrence of Bisphenol A and its analogues in some foodstuff marketed in Europe. Food Chem Toxicol. 2019;131:110575.
57. Mercogliano R, Santonicola S. Investigation on bisphenol A levels in human milk and dairy supply chain: a review. Food Chem Toxicol. 2018;114:98–107.
58. Sifakis S, et al. Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. Environ Toxicol Pharmacol. 2017;51:56–70.
59. Martin EM, Fry RC. Environmental influences on the epigenome: exposure- associated DNA methylation in human populations. Annu Rev Public Health. 2018;39:309–33.
60. Strakovsky RS, Schantz SL. Using experimental models to assess effects of bisphenol A (BPA) and phthalates on the placenta: challenges and perspectives. Toxicol Sci. 2018;166 (2):250–68.
61. Tomza-Marciniak A, et al. Effect of bisphenol A on reproductive processes: a review of in vitro, in vivo and epidemiological studies. J Appl Toxicol. 2018;38(1):51–80.
62. Ejaredar M, et al. Bisphenol A exposure and children's behavior: a systematic review. J Expo Sci Environ Epidemiol. 2017;27(2):175–83.
63. Rancière F, et al. Bisphenol A and the risk of cardiometabolic disorders: a systematic review with meta-analysis of the epidemiological evidence. Environ Health. 2015;14:46.
64. Rahmani S, et al. Bisphenol A: what lies beneath its induced diabetes and the epigenetic modulation? Life Sci. 2018;214:136–44.
65. Nadal A, et al. Endocrine-disrupting chemicals and the regulation of energy balance. Nat Rev Endocrinol. 2017;13(9):536–46.
66. Thent ZC, Froemming GRA, Muid S. Bisphenol A exposure disturbs the bone metabolism: an evolving interest towards an old culprit. Life Sci. 2018;198:1–7.
67. Xie MY, et al. Exposure to bisphenol A and the development of asthma: a systematic review of cohort studies. Reprod Toxicol. 2016;65:224–9.
68. Wang Z, Liu H, Liu S. Low-dose bisphenol A exposure: a seemingly instigating carcinogenic effect on breast cancer. Adv Sci (Weinh). 2017;4(2):1600248.
69. Dempsey JL, Cui JY. Long non-coding RNAs: a novel paradigm for toxicology. Toxicol Sci. 2017;155(1):3–21.
70. Doshi T, D'Souza C, Vanage G. Aberrant DNA methylation at Igf2-H19 imprinting control region in spermatozoa upon neonatal exposure to bisphenol A and its association with post implantation loss. Mol Biol Rep. 2013;40(8):4747–57.
71. Kabir ER, Rahman MS, Rahman I. A review on endocrine disruptors and their possible impacts on human health. Environ Toxicol Pharmacol. 2015;40(1):241–58.
72. Giakoumelou S, et al. The role of infection in miscarriage. Hum Reprod Update. 2016;22 (1):116–33.
73. Sharma R, et al. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol. 2013;11:66.
74. Jaworek H, et al. Prevalence of human papillomavirus infection in oocyte donors and women treated for infertility: an observational laboratory-based study. Eur J Obstet Gynecol Reprod Biol X. 2019;4:100068.
75. Subtil D, et al. Early clindamycin for bacterial vaginosis in pregnancy (PREMEVA): a multicentre, double-blind, randomised controlled trial. Lancet. 2018;392(10160):2171–9.
76. Donders GG, et al. Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. BJOG. 2009;116 (10):1315–24.
77. Perino A, et al. Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes. Fertil Steril. 2011;95(5):1845–8.

78. Spandorfer SD, et al. Prevalence of cervical human papillomavirus in women undergoing in vitro fertilization and association with outcome. Fertil Steril. 2006;86(3):765–7.
79. Gomez LM, et al. Placental infection with human papillomavirus is associated with spontaneous preterm delivery. Hum Reprod. 2008;23(3):709–15.
80. Pfeifer C, Bunders MJ. Maternal HIV infection alters the immune balance in the mother and fetus; implications for pregnancy outcome and infant health. Curr Opin HIV AIDS. 2016;11 (2):138–45.
81. Bunders MJ, et al. Fetal exposure to HIV-1 alters chemokine receptor expression by CD4+T cells and increases susceptibility to HIV-1. Sci Rep. 2014;4:6690.
82. Deeks SG, Tracy R, Douek DC. Systemic effects of inflammation on health during chronic HIV infection. Immunity. 2013;39(4):633–45.
83. Janier M, et al. 2014 European guideline on the management of syphilis. J Eur Acad Dermatol Venereol. 2014;28(12):1581–93.
84. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin Microbiol Rev. 2002;15(4):680–715.
85. Baudin M, et al. Association of Rift Valley fever virus infection with miscarriage in Sudanese women: a cross-sectional study. Lancet Glob Health. 2016;4(11):e864–71.
86. Uchide N, et al. Possible roles of proinflammatory and chemoattractive cytokines produced by human fetal membrane cells in the pathology of adverse pregnancy outcomes associated with influenza virus infection. Mediat Inflamm. 2012;2012:270670.
87. Littauer EQ, Esser ES. H1N1 influenza virus infection results in adverse pregnancy outcomes by disrupting tissue-specific hormonal regulation. PLoS Pathog. 2017;13(11):e1006757.
88. ACMSF. Report on risk profile in relation to toxoplasma in the food chain. Acta Acad Med Cpaf. 2012;7(7):49–61, illust.
89. Alvarado-Esquivel C, et al. Miscarriage history and toxoplasma gondii infection: a cross-sectional study in women in Durango City, Mexico. Eur J Microbiol Immunol (Bp). 2014;4 (2):117–22.
90. Vado-Solís IA, et al. Toxoplasma gondii presence in women with spontaneous abortion in Yucatan, Mexico. J Parasitol. 2013;99(2):383–5.
91. Lambalk CB, et al. GnRH antagonist versus long agonist protocols in IVF: a systematic review and meta-analysis accounting for patient type. Hum Reprod Update. 2017;23(5):560–79.
92. Easterlin MC, et al. Association of team sports participation with long-term mental health outcomes among individuals exposed to adverse childhood experiences. JAMA Pediatr. 2019;173(7):681–8.
93. Ångerud K, et al. Adverse childhood experiences and depressive symptomatology among pregnant women. Acta Obstet Gynecol Scand. 2018;97(6):701–8.
94. Field T. Prenatal depression effects on early development: a review. Infant Behav Dev. 2011;34(1):1–14.
95. Young-Wolff KC, et al. Adverse childhood experiences and mental and behavioral health conditions during pregnancy: the role of resilience. J Womens Health (Larchmt). 2019;28 (4):452–61.
96. Maconochie N, et al. Risk factors for first trimester miscarriage--results from a UK-population-based case-control study. BJOG. 2007;114(2):170–86.
97. Master JS, et al. Low female birth weight and advanced maternal age programme alterations in next-generation blastocyst development. Reproduction. 2015;149(5):497–510.
98. Ge ZJ, et al. Oocyte ageing and epigenetics. Reproduction. 2015;149(3):R103–14.
99. Gaskins AJ, et al. Association of fecundity with changes in adult female weight. Obstet Gynecol. 2015;126(4):850–8.
100. Steegers-Theunissen RP, et al. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. Hum Reprod Update. 2013;19 (6):640–55.

101. American College of Obstetricians and Gynecologists. ACOG Committee opinion no. 650: physical activity and exercise during pregnancy and the postpartum period. Obstet Gynecol. 2015;126(6):e135–42.
102. Terzioglu F. Investigation into effectiveness of counseling on assisted reproductive techniques in Turkey. J Psychosom Obstet Gynaecol. 2001;22(3):133–41.
103. Mutsaerts MA, et al. The influence of maternal and paternal factors on time to pregnancy--a Dutch population-based birth-cohort study: the GECKO Drenthe study. Hum Reprod. 2012;27 (2):583–93.
104. Berga S, Naftolin F. Neuroendocrine control of ovulation. Gynecol Endocrinol. 2012;28 (Suppl 1):9–13.
105. Schliep KC, et al. Perceived stress, reproductive hormones, and ovulatory function: a prospective cohort study. Epidemiology. 2015;26(2):177–84.
106. Rockwell LC, Keyes LE, Moore LG. Chronic hypoxia diminishes pregnancy-associated DNA synthesis in Guinea pig uteroplacental arteries. Placenta. 2000;21(4):313–9.
107. Gonzales GF, Ortiz I. Age at menarche at sea level and high altitude in Peruvian women of different ethnic background. Am J Hum Biol. 1994;6(5):637–40.
108. Crognier E, Villena M, Vargas E. Reproduction in high altitude Aymara: physiological stress and fertility planning? J Biosoc Sci. 2002;34(4):463–73.
109. Ghosh D, Kumar R, Pal K. Individual variation in response to simulated hypoxic stress of rats. Indian J Exp Biol. 2012;50(10):744–8.
110. Dosek A, et al. High altitude and oxidative stress. Respir Physiol Neurobiol. 2007;158 (2–3):128–31.
111. Lorca RA, et al. High altitude reduces no-dependent myometrial artery vasodilator response during pregnancy. Hypertension. 2019;73(6):1319–26.
112. De Fleurian G, et al. Occupational exposures obtained by questionnaire in clinical practice and their association with semen quality. J Androl. 2009;30(5):566–79.
113. Tielemans E, et al. Paternal occupational exposures and embryo implantation rates after IVF. Fertil Steril. 2000;74(4):690–5.
114. Lancranjan I, et al. Reproductive ability of workmen occupationally exposed to lead. Arch Environ Health. 1975;30(8):396–401.
115. Xu M, et al. An investigation of reproductive health and related influencing factors in female staff in six industries in seven provinces in China. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2016;34(12):924–7.

Part II
Female Reproductive Processes and Diseases

Chapter 2
Introduction of Female Reproductive Processes and Reproductive Diseases

Jiajia Zhang, Jiao Li, and Jie Yan

Abstract The female reproductive process is very complicated, including multiple processes. Each process is different and plays a vital role in reproduction. If some reproductive diseases occur, these processes will be abnormal, causing infertility problem. In this Chapter, we will describe the female reproductive process and their corresponding reproductive diseases.

Keywords Female reproductive process · Reproductive diseases · Endometrium · Ovulation · Fertilization

2.1 Female Reproductive Processes

2.1.1 The Female Reproductive System

The female reproductive system includes the internal and external genitalia. The external genitalia, usually the vulva, includes the mons pubis, labia majora, labia minora, clitoris, and the vaginal vestibule. The internal genitalia are located primarily in the pelvic cavity, including the vagina, uterus, fallopian tubes, and ovaries, the latter three are the main organs that affect the female reproductive process during the reproductive period.

1. The uterus is the organ that gestates offspring and ensues menstruation, it consists of the cervix and the uterine body. The uterine body is composed of the myometrium and the endometrium, which changing periodically with the influence of ovarian sex hormone, shedding to form menstruation.

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2. Fallopian tubes are tubes connected to the uterus, near the uterine horn, distal free in the abdominal cavity, adjacent to the ovary, to provide a place for ova and sperm to combine, and transfer oosperm to the uterine cavity.
3. The ovary is regulated by the central nervous system, which regulates and interacts with the endocrine system, called the hypothalamic-pituitary-ovarian axis. Under its control, the ovary periodically ovulates with cyclically changes of ovarian sex hormone secretion. Therefore, the ovary has both reproductive and endocrine functions.

The complete pregnancy process includes fertilization, transportation, and implantation. The embryo after implantation develops into a fetus in the uterus, and finally the fetus and its appendages are delivered from the uterus.

2.1.2 Cyclic Change of Endometrium

With the release of central negative feedback inhibition, GnRH (Gonadotropin-releasing hormone) begins to release in a pulsatile fashion, and women enter puberty and begin to experience secondary sexual characteristics such as breast germination and menarche, and gradually acquire mature reproductive capacity. The regular menstruation is the mark that female reproductive function matures. The endometrium is divided into the functional layer and the basal layer. The functional layer is the site of embryo implantation, which is regulated by ovarian hormones and periodically proliferates, secretes, and sheds. According to menstrual cycle, the endometrium is divided into three periods: proliferation phase, secretion phase, and menstrual phase.

2.1.2.1 Proliferation Phase

Corresponding to follicular phase of ovarian cycle, about 5~14 days of menstrual cycle, in the role of estrogen secreted by the ovary, the endometrial thickness increases, the epithelial, glandular, interstitial, and vascular proliferate.

2.1.2.2 Secretory Phase

Corresponding to the luteal phase in the ovarian cycle, which is about 15~28 days of the menstrual cycle. After ovulation, the ovarian luteal phase secretes progesterone and estrogen, which causes the endometrial glands to grow and bend, the stroma become loose and edematous, the blood vessels to continue to increase, the endometrium to continue to thicken, and it is rich in glycogen and other nutrients, conducive to the implantation of fertilized ova.

2.1.2.3 Menstrual Period

Corresponding to the ovarian cycle in the early follicles stage, progesterone and estrogen withdrawal, endometrial layer collapses and falls off, leading to the emergence of menarche.

2.1.3 Ovulation

2.1.3.1 Primordial Follicles

As early as in the gonad of 16-weeks female embryo, the follicle begins to form until 6 months after birth. It is composed of a primary oocyte in meiosis and a single layer of granular cells surrounding it, which is called primordial follicle. The primordial follicle is the only form of oocyte storage. There are about two million primordial follicles at birth, and they are declining in childhood, leaving about 300,000 at puberty.

2.1.3.2 Follicular Maturation

After puberty, approximately 3–10 antral follicles with a diameter of 0.5 mm were collected and selected in each menstrual cycle under the stimulation of gonadotropin, and only one dominant follicle could reach the final maturation and expel the oocyte. The rest of the follicles degenerate spontaneously, called follicular atresia.

2.1.3.3 Ovulation

Ovulation is the process by which the oocyte and its surrounding cumulus granulosa cells are discharged together.

The secretion of estradiol from mature ova reaches a peak value and produces a positive feedback regulation to the hypothalamus, which induces a large amount of GnRH release in the hypothalamus, then causes the pituitary to release gonadotropin and produces LH/FSH peak. LH peak is a reliable indicator of impending ovulation, appearing 36 h before the follicle ruptures. The LH peak stimulated primary oocyte to complete its first meiotic division, formed secondary oocyte, and stimulated the collagen layer of follicle wall to decompose and the formation of micropores. Menstruation occurs about 14 days after ovulation. Ovulation can occur alternately on both ovaries or on one ovary continuously.

2.1.3.4 Corpus Luteum

After ovulation, the walls of the follicles collapse, the granulosa cells and the endothecium cells of the follicles invade inwards and are surrounded by the outer membrane of the follicles of the connective tissue to form the corpus luteum.

If not fertilized, the luteal cells atrophy and become smaller, connective tissue and fibroblasts invade the corpus luteum, the tissue is fibrotic and appears white, the luteal gradually reduced to white body; if fertilized, the luteal cells are stimulated by hCG secreted by the embryonic trophoblastic cells to increase, and form pregnancy corpus luteum, which secret steroid hormones to maintain pregnancy. This process continues throughout the early pregnancy. The corpus luteum of pregnancy degenerates gradually after the placenta forms and secretes steroid hormones.

2.1.4 Fertilization

The sperm and the secondary oocyte (ovarian output) meet and fuse in the fallopian tube to form the oosperm.

Fertilization occurs within 12 h after ovulation. Sperm enters the vagina, through the cervix, uterine into the oviduct lumen. During this process, the chemical composition of acrosome surface changed, which decreased the stability of acrosome membrane and capacitated sperm. When the sperm and oocyte meet in the ampulla of uterine tube, the sperm head breaks up the outer membrane of the parietal body, releasing the acrosomal enzyme, which dissolves the corona radiata and zona pellucida (acrosome reaction) on the surface of the oocyte, allowing the sperm to enter and fuse with the oocyte to form an oosperm. When the sperm head contacts oocyte, the oocyte zona pellucida structure changes to prevent other sperm from entering into the same oocyte. Then the second meiosis of the oocyte is completed immediately. With the gradual fusion of female pronucleus and male pronucleus, chromosomes gradually mix to form a fertilized egg. It takes about 24 h to complete the whole fertilization process.

2.1.5 Transportation

Thirty hours after fertilization, the fertilized egg moves toward the uterine cavity under the combined action of oviduct peristalsis and fallopian tube epithelial cilia oscillation, at the same time carries on the mitosis, gradually forms the multicellular dividing ball, the mulberry embryo, and the blastocyst. On the fourth day after fertilization, the blastocyst entered the uterine cavity.

2.1.6 Implantation

The uterus has a window period to allow implantation of fertilized ova, that is, when the pregnant woman has enough progesterone, the blastocyst and endometrium develop synchronously and coordinately. The implantation process is divided into three stages: first, the zona pellucida disappears from the surface of blastocyst, and the blastocyst contacts the endometrium with the end of its inner cell mass, which is called localization; second, the blastocyst surface differentiates into syncytiotrophoblast, which is called adhesion; and the last step is invasion, trophoblast cells penetrate into the endometrium, part of the muscular layer and blood vessels, and the blastocyst is completely embedded in the endometrium.

2.1.7 Placental Formation

After embryo implantation, the trophoblast cells in the implantation site divide and proliferate, forming part of the phyllostomas in contact with the basal decidua, which is the main structure of the placenta. It has the functions of substance exchange, defense, hormone synthesis, and immunity, and is an important organ to maintain the growth and development of fetus in uterus. If the placental function is abnormal, it will lead to abnormal development of the fetus during pregnancy and even pretermination of pregnancy.

Female reproductive process is a complex physiological process, which will be abnormal or even terminated when external factors or internal discordance occur in any part of the process. The common diseases that affect women's reproductive health are described as below.

2.2 Reproductive Diseases

Female reproductive process is a complex physiological process, which will be abnormal or even terminated when external factors or internal discordance occur in any part of the process. The common diseases affecting women's reproductive health are described as below.

2.2.1 Endometrium

2.2.1.1 Endometritis Diseases

Endometritis diseases, such as chronic endometritis, endometrial polyps, endometrial tuberculosis, and intrauterine adhesions, can lead to infertility.

2.2.1.2 Chronic Endometritis

Chronic endometritis (CE) is localized inflammation of the endometrium, characterized by plasma cell infiltration in the stroma of the endometrium, usually without significant clinical symptoms, and occurs in about 14%~39% of infertile women [1–3], the rate of unexplained infertility is 55.7% [4], 9.3%~57.8% in recurrent miscarriage [5, 6], and in some studies it is as high as 60% or more [7]. The cause of chronic endometritis is unknown, which may result in abnormal expression of adhesion molecules, cytokines, chemokines, and apoptotic proteins in endometrium, leading to damage of endometrial receptivity, thus affecting embryo implantation [8].

2.2.1.3 Endometrial Polyp

Endometrial polyp is a benign lesion of endometrial hyperplasia. The glands and stroma of the endometrium, with the blood vessels as the core, protrude from the endometrium, some may have irregular vaginal bleeding, and some may be asymptomatic, the detection rate of endometrial polyp is as high as 35% in infertile women [9], and the mechanism of its effect on reproductive function is not completely clear, which may be related to irregular uterine bleeding leading chronic inflammatory reaction of endometrium and mechanical blocking of sperm transport and the embryo's contact with the endometrium [10]. And it alters the internal environment required for embryo implantation, such as abnormalities in the estrogen and progesterone receptors that affect decidualization of the endometrium; and increases levels of matrix metalloproteinase and cytokines [11].

2.2.1.4 Tuberculosis of Endometrium

The incidence of TB infection has historically been high in economically backward countries and regions. The incidence of TB has increased since 2012, with seven million new TB cases globally in 2018, up from 600 in 2017 [12]. Endometrial tuberculosis is often secondary to tuberculosis in other parts of the lung, accounting for 50–80% of genital tuberculosis. Tuberculosis bacteria can cause inflammatory changes, some can cause intrauterine adhesions, serious endometrial tuberculosis

can even lead to uterine atresia and amenorrhea, about 47% of tuberculosis patients have infertility [13].

2.2.1.5 Atypical Hyperplasia of Endometrium

When ovulation disorders occur, the menstrual cycle appears disorder due to the abnormal hormone levels. Endometrium lose the regular progesterone protection, and exposure to continuous stimulation of estrogen. As a result, endometrium may appear hyperplasia and even malignant transformation, which is not conducive to embryo implantation. The rate of endometrial dysplasia and endometrial cancer is 0.3%~0.39% in infertile women, which may lead to hyperplasia and malignant transformation [14, 15]. Obesity, persistent anovulatory, and endocrine disorder are the high-risk factors of the disease. Such diseases not only affect the reproductive process, but also have a serious impact on health.

2.2.2 Ovulation

The release of high-quality ova from the ovaries is a necessary condition for pregnancy. When a woman has polycystic ovary syndrome, hyperprolactinemia, ovarian dysfunction, or advanced age, failure to ovulate or poor oocyte quality can be a main cause of infertility.

2.2.2.1 PCOS

Polycystic ovary syndrome (PCOS) is characterized by high androgen levels, anovulatory and polycystic ovarian changes, and is often related to insulin resistance or obesity. The 2003 Rotterdam Standard is still the internationally recognized standard for diagnosing PCOS. The diagnosis of PCOS is based on two of the following three criteria: Oligo-and/or anovulation; clinical and/or biochemical evidence of hyperandrogenemia; and polycystic ovaries on ultrasound, with an incidence of 6%~16.6% in women of childbearing age [16]. PCOS is the most common cause of infertility, about 1/3 of infertile women exist, accounting for 80%~90% of anovulatory infertility [17].

In addition, PCOS women often have hyperinsulinemia/insulin resistance, high androgen levels, obesity, high lutropin alfa levels, hyperhomocysteinemia, and prethrombotic status, and these are high-risk factors for early abortion [18–20].

And PCOS has a high risk of pregnancy complications, such as hypertension, pre-eclampsia, and gestational diabetes. In Stefano's study, women with PCOS had a three- to fourfold increased risk of gestational hypertension and pre-eclampsia, and a threefold increased risk of gestational diabetes, the chance of premature birth doubles [21].

2.2.2.2 Hyperprolactinemia

Hyperprolactinemia, just as its name implies, is a disease with high serum prolactin levels. The clinical definition of HPRL is as follows: The level of fasting serum prolactin 2 h after waking up in the morning is higher than 25 ng/mL (1 ng/mL = 0.0455 nmol/L) in women, 20 ng/mL in men. The most common cause of hyperprolactinemia is pituitary prolactinoma. In addition, some other diseases that causes prolactin elevation by nerve feedback. For example, in hypothalamic diseases, the secretion of prolactin inhibitory factor is affected by tumor, which leads to the increase of pituitary prolactin. When hypothyroidism occurs, thyrotropin releasing hormone is increased, reflex stimulating pituitary prolactin secretion is increased; and long-term use of antipsychotic drugs also can cause prolactin to rise prolactin inhibitory factor is affected by tumor, which leads to the increase of pituitary prolactin. When hypothyroidism occurs, thyrotropin releasing hormone is increased, reflex stimulating pituitary prolactin secretion is increased; and long-term use of antipsychotic drugs also can cause prolactin to rise [22].

With the increase of prolactin, the secretion of LH and FSH in pituitary was inhibited, and the function of ovarian secretion was disturbed periodically. Thus patients with menstrual disorders, libido decline, ovulation disorders, luteal insufficiency result in infertility [23, 24].

2.2.2.3 Ovarian Dysfunction

The number of follicles in women's ovaries decreases with age, as does the quality of their ova [25]. Chromosome abnormalities and aneuploidy also increase, with more than 50% of women over 40 suffering from ovarian failure [26]. In addition to age, autogenetic factors, ovarian cysts, ovarian surgery, infections, autoimmune diseases, and adverse environmental conditions all have direct or indirect effects on ovarian function.

2.2.2.4 Ovarian Cyst

The most common ovarian cyst in infertility women is endometriosis cyst, also known as endometriosis, in which endometrial glands and stroma occur in the ovaries, as well as in the fallopian tubes, pelvic peritoneum, and uterosacral ligaments. By affecting the development of follicles, oocyte quality, and corpus luteum function, ovarian reserve decreased [27], endometriosis can also lead to pelvic, fallopian tube adhesion, resulting in oocyte, sperm, and fertilized egg transport barriers.

At the same time [28], endometriosis increased the volume of fluid in the pelvic cavity and increased the concentration of inflammatory mediators, prostaglandins, interleukin-1, tumor necrosis factor, and proteases, which affect the ova [29], thus causing a decline in female fertility. About 30%~50% of patients with endometriosis have difficulty conceiving [30], and 17%~47% of infertile women have endometriosis [31].

2.2.3 Fertilization and Transport

The fallopian tube is an important place for sperm and oocyte to combine, and it is also an important organ for transporting fertilized ova to the uterine cavity. Abnormal fallopian tube, sperm and oocyte encounter obstruction, or fertilization egg transport obstacles, can lead to infertility or ectopic pregnancy. Among the causes of female infertility, the incidence of fallopian factors ranged from 25%~35% [32].

The most common related disease is pelvic inflammatory disease (PID), which is usually caused by unsanitary sex or post-abortion infection, including pelvic adhesions, salpingitis, tubal obstruction, hydrosalpinx, or formation of a tubal cyst, can affect the fimbriae of uterine tube and oviduct to ova, fertilized ova transport function; One third of the most common pathogens are Neisseria gonorrhoeae and chlamydia trachomatis, one third are mycoplasma and anaerobic (bacteroides, clostridium, and streptococcus) infections, and the rest include hemophilus influenzae, group A streptococcus, actinomycetes, and other microorganisms [33, 34].

The pathogens spread from the lower genital tract to the upper genital tract through the cervix and uterine cavity, and then into the fallopian tube, leading to mucosal edema, and eventually adhesion.

In addition, the inflammatory changes of other pelvic organs such as appendicitis perforation can also cause tubal adhesion, obstruction. Pelvic tuberculosis, especially tubal tuberculosis, is also one of the vital causes of tubal infertility, 90%~95% of tubal tuberculosis women with infertility [35].

Adhesion after surgery can also lead to tubal adhesion, which affects the function of the fallopian tube. Some studies show that the incidence of adhesion after pelvic and abdominal surgery is 90%~95% [36].

Moreover, congenital malformation of genital tract, such as oviduct is congenital absent, oviduct is too long and thin, oviduct muscle layer is weak, resulting in oviduct diverticulum, oviduct accessory fimbria, and other structural abnormalities [37, 38], may also be the high risk factors of infertility and ectopic pregnancy.

2.2.4 Uterus Factor

The uterus is an important organ to produce offspring. In addition to the abnormalities of the endometrium itself mentioned above, the myometrium also affects the reproductive process.

2.2.4.1 Uterine Fibroid

Uterine leiomyoma is the most usual benign tumor in female, and the incidence rate is about 25%~30%. For some uterine fibroids without any clinical symptoms, the actual incidence rate may be higher. In infertile women the incidence rate is 5%

~10% [39]. In particular, submucous myoma, which has a special position and protrudes into the uterine cavity, affects the growth and development of embryo implantation fetal sac [39, 40], but the effect of myoma between muscle wall and subserosa on fertility is still controversial.

The effect of hysteromyoma on fertility is not only the mechanical change of uterine cavity shape and the change of endometrial blood flow to affect embryo implantation, endometrial receptivity may also be influenced by biochemical signals: Uterine fibroid can induce elevated levels of collagen, fibronectin, laminin, and proteoglycan and can also reduce the expression of HOX genes, hemophilia inhibitors, and integrin β3 and secrete TGF β, which affect the receptors for BMP 1 and BMP 2, all of these may cause endometrial receptivity damage [41, 42].

2.2.4.2 Adenomyosis

Adenomyosis is one kind disease where endometrial glands and stroma invading into the myometrium. It usually occurs in women more than 30 years old, and occurs in up to 50% of infertile women, often with endometriosis [43]. Ectopic endometrium was diffusely growing in myometrium and was enlarged evenly. The patients often have gradual aggravation dysmenorrhea, menorrhagia, and menstrual period extension.

Adenomyosis affects fertility in the following ways: induce local inflammation; affect sperm transport by altering endometrial peristalsis initiated by the uterine junctional zone; change endometrial function and receptivity [44].

2.2.4.3 Congenital Malformation of Uterus

Uterine congenital structure abnormalities have a great impact on reproductive health. Some patients also have abnormal development of vagina and vulva. Uterine septum is the commonest malformation in female of childbearing age, and its incidence rate is 2%~3% [45]. The cases of congenital uterine malformation reported in the literature are uterus septum (34.9%), uterus bicornis (26%), arcuate uterus (18.3%), uterus unicornis (9.6%), and uterus didelphys (8.4%) [46], the rate of pregnancy loss of incomplete septum was 44.3% [47].The cause of uterine dysplasia is unknown, but exposure to diethylstilbestrol is one of the causes of the “T” shape of the uterus.

2.2.4.4 Acquired Factor

Intrauterine adhesion is the main reproductive abnormality caused by acquired factors. Intrauterine adhesion is the destruction of the endometrial basement layer, which leads to endometrial loss, adhesion, scar or even uterine cavity deformation. Its inducement usually was abortion, curettage, and other mechanical injury, also can be

caused by non-specific inflammation or tuberculosis. The more severe the intrauterine adhesions, the more serious the damage to the endometrium, and the damage to the endometrium directly determines the menstrual condition and reproductive prognosis [48]. The more severe the intrauterine adhesions, the lower the clinical pregnancy rate. Roy et al. made statistical analysis on the pregnancy of the patients after uterine cavity adhesion separation. The total pregnancy rate was 40.4%, and the pregnancy rates of the patients with mild, moderate, and severe adhesion were 58%, 30%, and 33.3%, respectively; the full-term delivery rate was 94.4%, 83.3%, and 66.6%, respectively [49]. The pregnancy rate of 357 cases of intrauterine adhesions treated by Chen et al. was 48.2%. The pregnancy rates of mild, moderate, and severe cases were 60.7%, 53.4%, and 25%, respectively [50]. The rate of spontaneous abortion in patients with intrauterine adhesions was about 20%, higher than 12% in the general population [51].

2.2.5 Immune and Endocrine Factors

2.2.5.1 Luteal Phase Defect, LPD

A certain level of progesterone is one of the important conditions for maintaining pregnancy. The secretion of progesterone in the early stage is completed by the ovarian corpus luteum. After 10 weeks of pregnancy, the placenta gradually forms and begins to secrete various hormones to maintain pregnancy, and then the function of the ovarian corpus luteum is gradually replaced by the placenta. If the function of ovarian corpus luteum is insufficient, the production of progesterone to maintain the pregnancy in early pregnancy will not be enough, and early pregnancy loss may occur. Between 25% and 40% of recurrent abortion is associated with luteal insufficiency, and about 3%~4% of infertile women also have luteal insufficiency [52]. In women with endometriosis and insulin resistance, luteal dysfunction is particularly evident [53, 54].

2.2.5.2 Hypothyroidism

Hypothyroidism, especially subclinical hypothyroidism, is common in female childbearing age with an incidence of 2%~3% in pregnant women, and even 40.0% and 15.4% in those with ovarian failure and ovulation disorder [55]. The mechanism may be that hypothyroidism has a negative feedback effect on the pituitary-thyroid axis, and the pituitary secretes thyroid stimulating hormone (TSH) level, leading to the increase of TRH and the decrease of dopamine secretion, thus promoting the secretion of prolactin (PRL). Thus hypothyroidism is often accompanied by hyperprolactinemia, which affects the menstrual cycle and ovulation. During pregnancy, thyroid hormone is indispensable for fetal brain development and maturation. Fetus can only synthesize thyroid hormone in the late second trimester of pregnancy.

If hypothyroidism occurs, the risk of miscarriage, fetal neurological and intellectual dysplasia distinctly increases. Multiple studies have shown that the risk of miscarriage and preterm birth increases when thyroid autoantibodies are positive [56].

2.2.5.3 Hyperthyroidism

The incidence of hyperthyroidism in pregnancy is low, about 0.05%~1.7% [57]. It is often found due to hyperemesis gravidarum, the mother with hyperthyroidism is in a high metabolic state and the placental supply of energy is reduced, which can lead to maternal pre-eclampsia, fetal growth retardation, fetal tachycardia, congestive heart failure, and even fetal death in utero. Thyrotropin receptor antibody (TRAb) can pass through the placenta. Monitoring of TRAb titers in high-risk patients during early and middle pregnancy can effectively predict the occurrence of fetal hyperthyroidism [58].

2.2.5.4 Antiphospholipid Syndrome

Pregnancy is a kind of allotransplantation. The embryo, as an allogeneic, is accepted by the maternal immune system. Therefore, pregnancy is also a process in which the immune rejection is suppressed. When the mother has abnormal immune function, the maintenance of the pregnancy process will also be impaired.

Antiphospholipid syndrome is one of the most recognized autoimmune diseases that affect pregnancy. It is a non-inflammatory autoimmune disease, where clinical manifestations were arterial thrombosis, venous thrombosis, recurrent miscarriage, and thrombocytopenia, and it is characterized by the production of a huge number of antiphospholipid antibodies (aPL), including lupus anticoagulant antibodies (LA), anti-cardiolipin antibodies (aCL), and anti-β2 glycoprotein I antibodies (β2GPI).

These patients often have recurrent loss of pregnancy during early pregnancy, and the possible mechanisms include protein C axis inhibition, increased thrombin production, complement imbalance, endothelial cell activation, and platelet activation. In the early stage of pregnancy, antiphospholipid syndrome can lead to the formation of thrombus in decidua, which can affect the blood supply to the embryo, resulting in embryonic development abnormally and abortion [59]. The high expression of β2GPI on the placenta and decidual surface in the middle and third trimester of pregnancy can lead to thrombosis and even serious complications such as fetal growth restriction, pre-eclampsia, and even intrauterine fetal death [60, 61].

2.3 Conclusion

In conclusion, both inflammatory and neoplastic diseases of the reproductive system and systemic endocrine and metabolic diseases seriously influence female reproductive health. It is suggested that women who have reproductive requirements should not only be examined for physical diseases with symptoms, but also improve their reproductive health from living habits and environment in order to achieve the goal of eugenics and good breeding.

References

1. Polisseni F, Bambirra EA, Camargos AF. Detection of chronic endometritis by diagnostic hysteroscopy in asymptomatic infertile patients. Gynecol Obstet Investig. 2003;55:205–10.
2. Kasius JC, Fatemi HM, Bourgain C, et al. The impact of chronic endometritis on reproductive outcome. Fertil Steril. 2011;96:1451–6.
3. Kasius JC, Broekmans FJM, Sie-Go DMDS, et al. The reliability of the histological diagnosis of endometritis in asymptomatic IVF cases: a multicenter observer study. Hum Reprod. 2012;27:153–8.
4. Cicinelli E, Matteo M, Trojano G, et al. Chronic endometritis in patients with unexplained infertility: prevalence and effects of antibiotic treatment on spontaneous conception. Am J Reprod Immunol. 2018;79:e12782.
5. Kitaya K. Prevalence of chronic endometritis in recurrent miscarriages. Fertil Steril. 2011;95 (3):1156–8.
6. Zolghadri J, Momtahan M, Aminian K, et al. The value of hysteroscopy in diagnosis of chronic endometritis in patients with unexplained recurrent spontaneous abortion. Eur J Obstet Gynecol Reprod Biol. 2011;155(2):217–20.
7. Cicinelli E, Matteo M, Tinelli R, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Hum Reprod. 2015;30:323–30.
8. Kitaya K, Matsubayashi H, Takaya Y, et al. Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure. Am J Reprod Immunol. 2017;78(5):e12719.
9. Check JH, Bostick Smith CA, Choe JK, et al. Matched controlled study to evaluate the effect of endometrial polyps on pregnancy and implantation rates following in vitro fertilization-embryo transfer (IVF-ET). Clin Exp Obstet Gynecol. 2011;38(3):206–8.
10. Yanaihara A, Yorimitsu T, Motoya H, et al. Location of endometrial polyp and pregnancy rate in infertility patients. Fertil Steril. 2008;90(1):180–2.
11. Richlin SS, Ramachandran S, Shanti A, et al. Glycodelin levels in uterine flushings and in plasma of patients with leiomyomas and polyps: implications for implantation. Hum Reprod. 2002;17(10):2742–7.
12. Harding E. WHO global progress report on tuberculosis elimination. Lancet Respir Med. 2019;8(1):19.
13. Bazaz G, Maheshwari B, Lal N. Tuberculous endometritis: a clinicopathological study of 1000 cases. BJOG Int J Obstet Gynaecol. 1983;90(1):84–6.
14. Aytac TY, Bulent ZH, Deniz AO, et al. Prevalence of endometrial cancer or atypical hyperplasia diagnosed incidentally in infertility clinic. Am J Obstet Gynecol. 2018;219(5):503–5.

15. Kuribayashi Y, Nakagawa K, Sugiyama R, et al. Frequency of endometrial cancer and atypical hyperplasia in infertile women undergoing hysteroscopic polypectomy. J Obstet Gynaecol Res. 2017;43(9):1465–71.
16. Lauritsen MP, Bentzen JG, Pinborg A, et al. The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Mullerian hormone. Hum Reprod. 2014;29(4):791–801.
17. Balen AH. Polycystic ovary syndrome (PCOS). Obstet Gynaecol. 2017;19(2):119–29.
18. Tian L, Shen H, Lu Q, et al. Insulin resistance increases the risk of spontaneous abortion after assisted reproduction technology treatment. J Clin Endocrinol Metab. 2007;92(4):1430–3.
19. Banu J, Fatima P, Sultana P, et al. Association of infertile patients having polycystic ovarian syndrome with recurrent miscarriage. Mymensingh Med J. 2014;23(4):770–3.
20. Kazerooni T, Ghaffarpasand F, Asadi N, et al. Correlation between thrombophilia and recurrent pregnancy loss in patients with polycystic ovary syndrome: a comparative study. J Chin Med Assoc. 2013;76(5):282–8.
21. Stefano P, De WMA, Angela F, et al. Pregnancy complications in women with polycystic ovary syndrome. Hum Reprod Update. 2015;21(5):575–92.
22. Peuskens J, Pani L, Detraux J, et al. The effects of novel and newly approved antipsychotics on serum prolactin levels: a comprehensive review. CNS Drugs. 2014;28(5):421–53. https://doi.org/10.1007/s40263-014-0157-3.
23. Halbreich U, Kinon BJ, Gilmore JA, et al. Elevated prolactin levels in patients with schizophrenia: mechanisms and related adverse effects. Psychoneuroendocrinology. 2003;28(Suppl 1):53–67.
24. Capozzi A, Scambia G, Lello S, et al. Hyperprolactinemia: pathophysiology and therapeutic approach. Gynecol Endocrinol. 2015;31(7):506–10.
25. Faddy MJ, Gosden RG, Gougeon A, et al. Accelerated disappearance of ovarian follicles in mid-life: implications forecasting menopause. Hum Reprod. 1992;7(10):1342–6.
26. Ferranretti AP, La Marca A, Fauster BC, et al. ESHRE working group on poor ovarian response definition. ESHRE consensus on the definition of poor response to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26(7):1616–24.
27. Sanchez AM, Somigliana E, Vercellini P, et al. Endometriosis as a detrimental condition for granulosa cell steroidogenesis and development: from molecular alterations to clinical impact. J Steroid Biochem Mol Biol. 2016;155(Pt A):35–46.
28. Schenken RS, Asch RH, Williams RF, Hodgen GD. Etiology of infertility in monkeys with endometriosis: luteinized unruptured follicles, luteal phase defects, pelvic adhesions and spontaneous abortions. Fertil Steril. 1984;41:122–30.
29. Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. Fertil Steril. 2001;75:1–10.
30. Missmer SA, Hankison SE, Spiegelman D, Barbieri RL, Marshall LM, Hunter DJ. Incidence of laparoscopically confirmed endometriosis by demographic, anthropometric, and lifestyle factors. Am J Epidemiol. 2004;160:784–96.
31. Peterson CM, Johnstone EB, Hammoud AO, et al. Risk factors associated with endometriosis: importance of study population for characterizing disease in the ENDO study. Am J Obstet Gynecol. 2013;208(6):451.e1–e11.
32. Serafini P, Batzofin J. Diagnosis of female infertility. J Reprod Med. 1989;34:29–40.
33. Taylor-Robinson D, Jensen JS, Svenstrup H, et al. Difficulties experienced in defining the microbial cause of pelvic inflammatory disease. Int J STD AIDS. 2012;23(1):18–24.
34. Wang Y, Zhang Y, Zhang Q, et al. Characterization of pelvic and cervical microbiotas from patients with pelvic inflammatory disease. J Med Microbiol. 2018;67(10):1519–26.
35. Chowdhury NN. Overview of tuberculosis of the female genital tract. J Indian Med Assoc. 1996;94(9):345–346, 361.
36. Okabayashi K, Ashrafian H, Zacharakis E, et al. Adhesions after abdominal surgery: a systematic review of the incidence, distribution and severity. Surg Today. 2014;44(3):405–20.

37. Guan J, Watrelot A. Fallopian subtle pathology. Best Pract Res Clin Obstet Gynaecol. 2019;59:25–40.
38. Chen B, Yang C, Sahebally Z, et al. Unilateral ovarian and fallopian tube agenesis in an infertile patient with a normal uterus. Exp Ther Med. 2014;8(3):831–5.
39. Klatsky P, Tran N, Caughey A, et al. Fibroids and reproductive outcomes: a systematic review from conception to delivery. Am J Obstet Gynecol. 2008;198(4):357–66.
40. Sunkara S, Khairy M, El-Toukhy T, et al. The effect of intramural fibroids without uterine cavity involvement on the outcome of IVF treatment: a systematic review and metaanalysis. Hum Reprod. 2010;25(2):418–29.
41. Islam MS, Ciavattini A, Petraglia F, et al. Extracellular matrix in uterine leiomyoma pathogenesis: a potential target for future therapeutics. Hum Reprod Update. 2017;24(1):1–27.
42. Doherty LF, Taylor HS. Leiomyoma-derived transforming growth factor-b impairs bone morphogenetic protein-2–mediated endometrial receptivity. Fertil Steril. 2015;103:845–52.
43. Brosens JJ, de Souza NM, Barker FG. Uterine junctional zone: function and disease. Lancet. 1995;346:558–60.
44. Garavaglia E, et al. Adenomyosis and infertility: is there a causal link? Int J Reprod BioMed. 2015;13(6):327–36.
45. Rai R, Regan L. Recurrent miscarriage. Lancet. 2006;368:601–11.
46. Chan YY, Jayaprakasan K, Zamora J, et al. The prevalence of congenital uterine anomalies in unselected and high-risk populations: a systematic review. Hum Reprod Update. 2011;17 (6):761–71.
47. Gruszka M, Wilczyński J, Nowakowska D. Prevalence of uterine malformations and their impact on fertility. Ginekol Pol. 2012;83(7):517–21.
48. Bosteels J, Weyers S, Mol BW, et al. Anti-adhesion barrier gels following operative hysteroscopy for treating female infertility: a systematic review and meta-analysis. Gynecol Surg. 2014;11:113–27.
49. Roy KK, Baruah J, Sharma JB, et al. Reproductive outcome following hysteroscopic adhesiolysis in patients with infertility due to Asherman's syndrome. Arch Gynecol Obstet. 2010;281(2):355–61.
50. Chen L, Zhang H, Wang Q, et al. Reproductive outcome in patients with intrauterine adhesions following hysteroscopic adhesiolysis: experience from the largest women's hospital in China. J Minim Invasive Gynecol. 2017;24(2):299–304.
51. Panayotidis C, Weyers S, Bosteels J, et al. Intrauterine adhesions (IUA): has there been progress in understanding and treatment over the last 20 years? Gynecol Surg. 2009;6(3):197–211.
52. Bopp B, Shoupe D. Luteal phase defects. J Reprod Med. 1993;38(5):348–56.
53. Pittaway DE, Maxson W, Daniell J, et al. Luteal phase defects in infertility patients with endometriosis. Fertil Steril. 1983;39(5):712–3.
54. Beardsley RD, Holden JP. A novel definition of insulin resistance helps elucidate luteal phase defects. Fertil Steril. 2017;108(3):e250.
55. Abalovich M, Amino N, Barbour LA, et al. Management of thyroid dysfunction during pregnancy and postpartum: an endocrine society clinical practice guideline. Clin Endocrinol Metab. 2007;92(8 Suppl):S1–47.
56. Vissenberg R. Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. Hum Reprod Update. 2011;17(5):605–19.
57. Banerjee S. Thyroid disorders in pregnancy. J Assoc Physicians India. 2011;59(Suppl):32–4.
58. Alamdari S, Azizi F, Delshad H, et al. Management of hyperthymidism in pregnancy: comparison of recommendations of American thyroid association and endocrine society. J Thyroid Res. 2013;2013:878467.
59. Ippolito S, Meroni P, Koike T, et al. Obstetric antiphospholipid syndrome: a recent classification for an old defined disorder. Autoimmun Rev. 2014;5(1):1–8.

60. Meroni PL, Borghi MO, Grossi C, et al. Obstetric and vascular antiphospholipid syndrome: same antibodies but different diseases? Nat Rev Rheumatol. 2018;14(7):433–40.
61. Carp HJA, Shoenfeld Y. Recurrent spontaneous abortions in antiphospholipid syndrome: natural killer cells—an additional mechanism in a multi factorial process. Rheumatology. 2007;46(10):1517–9.

Part III
Effects of Environmental Factors on Reproductive Process

Chapter 3
The Influence of Environmental Factors on Ovarian Function, Follicular Genesis, and Oocyte Quality

Jiana Huang and Haitao Zeng

Abstract Endocrine-disrupting chemicals (EDCs) exist ubiquitously in the environment. Epidemiological data suggest that the increasing prevalence of infertility may be related to the numerous chemicals. Exposure to EDCs may have significant adverse impacts on the reproductive system including fertility, ovarian reserve, and sex steroid hormone levels. This chapter covers the common exposure ways, the origins of EDCs, and their effects on ovarian function, follicular genesis, and oocyte quality. Furthermore, we will review the origin and the physiology of ovarian development, as well as explore the mechanisms in which EDCs act on the ovary from human and animal data. And then, we will focus on the bisphenol A (BPA), which has been shown to reduce fertility and ovarian reserve, as well as disrupt steroidogenesis in animal and human models. Finally, we will discuss the future direction of prevention and solution methods.

Keywords Endocrine-disrupting chemicals (EDCs) · Bisphenol A (BPA) · Folliculogenesis · Oocyte · Steroidogenesis

3.1 The Common Exposure Ways, the Origins of EDCs, and their Effects on Ovarian Function, Follicular Genesis, and Oocyte Quality

Endocrine-disrupting chemicals (EDCs) are extrinsic chemicals that can interfere with the processes regulated by endogenous hormones. EDC was defined as "an exogenous chemical or mixture of chemicals that interferes with any aspect of hormone action"[1] in 2012 by the Endocrine Society. They emphasized that very low dose EDC exposures during the developmental stage might have potent and

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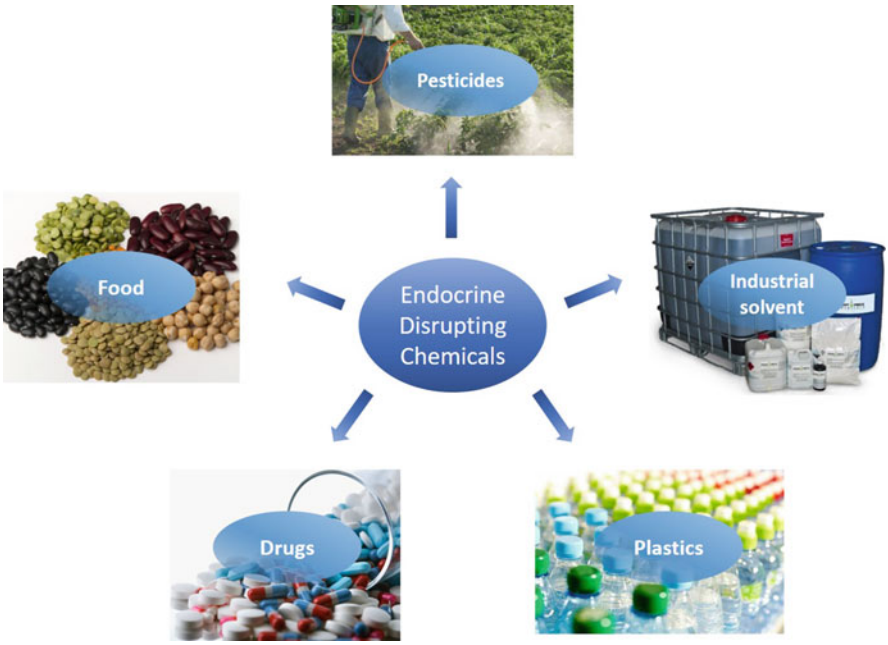

Fig. 3.1 Common exposure origins of endocrine disrupting chemicals

irreversible effects. The female reproductive system is regulated by hormones which means it would be the target of EDCs. Ovary is an important assurance for fertility and performs normal functions of oocyte/follicular quality, folliculogenesis, or steroidogenesis, which is also a target constitution assaulted by EDCs. As a result, EDCs exposure may disrupt folliculogenesis, oocyte quality as well as steroidogenesis.

EDCs are ubiquitous in the environment, and a recent report has indicated that there are more than 800 chemicals with endocrine-disrupting properties been used in daily life [2]. People and animals tend to exposure to EDCs by various routes, such as direct contact, inhalation, ingestion, maternal-fetal transfer, or intravenous administration [3]. Generally, there are two categories of EDCs: One is naturalistic EDCs such as phytoestrogen, genistein, and coumestrol found in natural food. The other one is synthetic EDCs which can be further divided into the following groups: Dioxins, polybrominated biphenyls (PBBs), and polychlorinated biphenyls (PCBs) that are found in industrial synthetic chemicals and their by-products; Bisphenol A (BPA) and phthalate in plastics; Methoxychlor (MXC) and dichlorodiphenyltrichloroethane (DDT) in pesticides; Vinclozolin in fungicide and diethylstilbestrol (DES) in some pharmaceutical agents [4] (Fig. 3.1).

Epidemiological data have suggested that EDCs may accumulate in human body and the environment. BPA and four phthalate metabolites (mono-(2-ethylhexyl) phthalate (MEHP), monomethyl phthalate (MMP), monoisobutyl phthalate (MiBP), monoethyl phthalate (MEP)) could be detected in nearly all 1016

participants aged 70[5]. A Spanish cohort study in 2011 discovered that phthalate and phenol were commonly found in the urine of young children and pregnant women, with the urinary concentrations higher in children than in pregnant women [6]. Research in 2015 indicated that organochlorine pesticides, 13.4% of which was MXC, could be detected in follicular fluid of women from central China [7]. Besides, BPA and its derivatives can be detected in serum from second-trimester umbilical cord [8], which indicates that EDCs can be passed from mother to fetus through placenta and concentrated in fetal body. Furthermore, EDCs are difficult to be eradicated by biodegradation. For instance, MXC is persistent in soil, and its residues are present even 18 months after the soil treatment with microorganisms [9].

EDCs are prone to expose and accumulate in human body, and they can be detected in people of all ages. In addition, the existence of EDCs in follicular fluid raises a concern that EDCs may affect the reproductive system and even cause epigenetic modification of gametes. What's more, the accumulation of EDCs in the environment is difficult to be eradicated by biodegradation, having a long-lasting impact on human health.

Researches have disclosed that EDCs adversely affect the ovary by disrupting folliculogenesis, oocyte development, and ovarian function. For instance, numerous studies have elucidated that EDCs interfered with folliculogenesis and oocyte development. Mixtures of EDCs (BPA, pesticides, phthalates, butylparaben, paracetamol, and UV-filters.) exposed to rats before puberty cause a significant reduction in primordial follicle quantities and plasma levels of prolactin [10]. Prenatal treatment of caiman latirostris with 17β-estradiol (E2), BPA, or atrazine (ATZ) can increase type III follicles, and treatment with BPA or E2 also presents higher proportioned multi-oocyte follicles [11]. Di(2-ethylhexyl) phthalate (DEHP) and MEHP can inhibit antral follicle growth via reducing estradiol production and decreasing the expression of cell cycle regulators [12]. DEHP has been proved to inhibit follicle growth and induce antral follicle atresia via dysregulation of cell cycle and apoptosis regulators [13]. Furthermore, EDCs have negative impacts on oocyte development. Female adult South African clawed toads (Xenopus laevis) that were exposed to tamoxifen (TAM) and methyldihydrotestosterone (MDHT) showed oocyte atresia in a previous study [14]. EDCs can disrupt the oocyte meiotic progression of in vitro cultured porcine oocyte cumulus complexes (OCC). BPA and 4-chloro-3-methyl phenol (CMP) exposure reduces numbers of oocytes undergoing germinal vesicle breakdown (GVBD) or reached metaphase II stage (MII) via meiotic maturation disturbance. Besides, BPA and CMP can reduce the synthesis of extracellular matrix (ECM) by altering the process of cumulus expansion [15].

Multiple studies have also consistently shown that EDCs exposure disrupted ovarian function. For example, the serum level of BPA, octylphenol (OP), and 4-nonylphenol (4-NP) is significantly elevated in precocious girls. Prepubertal exposure to EDCs including genistein, zearalenone, zeranol, and DES results in acceleration of puberty earlier onset, prolonged estrous cycle, and anovulatory period [16]. Besides, BPA and OP levels are positively correlated with the volume of the uterus and ovary [17]. Prenatal ewes exposure to mixture EDCs reduces ovarian reserve, greatly increasing the number of altered fetal ovarian genes and proteins [18]. Zama et al. [19] have suggested that transient exposure to MXC during

fetal and neonatal development results in ovarian dysfunction via significant hypermethylation in the ERβ promoter regions and increase of DNMT3B. Further studies have revealed that transient exposure to MXC results in epigenetic modification in ovaries via specific signaling pathways such as IGF-1 signaling, PTEN signaling, and rapid estrogen signaling [20].

3.2 The Origin and the Physiology of Ovarian Development, as well as Briefly Introduce the Mechanisms in which EDCs Act on the Ovary

Ovary is a complex organ that is responsible for gametogenesis and steroidogenesis. And ovary is one of the most important target organs of endocrine disruptor chemicals. The oogonia develop from the yolk sac and then migrate to the ovary, which are proliferated by mitosis until about gestation age 28–30 weeks, and then start meiosis. The oogonia differentiate into primary oocytes, which progress into the prophase of the first meiotic division and then become dormant until puberty. The number of oocytes comes to a climax around six to seven million at gestation age 25–28 weeks, which and then starts a steady decline because of atresia. There are approximately 700,000 to two million germ cells in the neonatal period and about 300,000 to 500,000 primordial follicles at the time of puberty. For females reaching 37 years old, the numbers of primordial follicles decline to 25,000. There are about only 1000 primordial follicles reserved for peri-menopausal women (Fig. 3.2). At the period of fetus and child, the follicles hardly reach maturation. After puberty, with the stimulation of gonadotropin secreted by the pituitary gland, there is a follicle maturation every month and then about 14 days before the onset of the next menstruation cycle. In a female's whole life, there are only 400 eggs successfully ovulated. And the rest of the follicles degenerate into atretic follicles. Follicles

Fig. 3.2 The change of gamete numbers along with age

consist of granulosa cells surrounding an oocyte. The follicular development in a continual process of four stages, including primordial follicles, primary follicles, secondary follicles, and mature follicles, has a series of structural and physiological changes (Table 3.1).

After introducing the basic physiology of ovary, we would briefly introduce the underlying mechanisms by EDCs working on ovary as follows.

EDCs are commonly identified as compounds that can interact with androgen or estrogen receptors and thus act as antagonists or agonists of endogenous hormones. EDCs disrupt the interference with hormone by mimicking or opposing actions and the hormonal and homeostatic systems [21]. In addition, the reproductive system is vulnerable to endocrine, especially during the early stage of life. Therefore, exposure to endocrine disruptors during development may lead to disease in children or adults and even to the next generations because of epigenetic modification [21].

3.2.1 Oxidative Stress

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, and hydroxyl radical are the by-products during natural oxygen metabolism, which can induce cellular damage and death and thus cause a wide range of diseases [22]. When ROS are excessively produced, or body clearance ability deficiency such as deficiency of antioxidants, oxidative stress will occur [23]. Oxidative stress is involved in ovarian toxicity caused by a variety of EDCs. It is well documented that ROS are involved in the initiation of apoptosis in follicular cells, poor quality of oocytes, and so on [23]. For instance, DEHP (10 μg/ml) has been proved to inhibit antral follicle growth by increasing ROS levels and reducing the expression and the activity of one of the critical antioxidant enzyme Cu/Zn superoxide dismutase (SOD) [1, 24]. MXC (1–100 μg/ml) can induce antral follicle atresia by decreasing the enzymatic activity and mRNA expression of antioxidant catalase Cu/Zn SOD1, glutathione peroxidase (GPX), and catalase [25]. In addition, neonatal exposure to EDCs (4-vinylcyclohexene diepoxide (VCD): 40–80 mg/kg/day, MXC: 50–100 mg/kg/day, and menadione: 7.5–15 mg/kg/day) can increase oocyte lipid peroxidation by ROS and thus induce permanent oocyte damage [26].

3.2.2 Disturbance of Steroidogenesis

Ovary is essential for the synthesis of steroid hormones. The "two-cell, two-gonadotropin" theory proposed by Armstrong et al. in 1979 demonstrates that granulosa cells express follicle-stimulating hormone (FSH) receptors, which stimulate aromatase activity, while theca cells possess luteinizing hormone (LH) receptors which stimulate androgen synthesis [27, 28]. During the process of ovarian steroidogenesis, LH stimulates the activation of 17α-hydroxylase to convert cholesterol and

Table 3.1 Characteristics of follicles in different stages

		Primordial follicle	Primary follicle	Secondary follicle	Antral follicle	Growing follicle		Preovulatory follicle	Corpus luteum
						Early	Late		
Diameter (mm)		0.03–0.06	>0.06	<0.12	>0.2	7–10	>10	16–20	–
Granulosa cell	Morphology	Simple squamous GC	Simple cuboidal GC	$<6*10^2$ cuboidal GC	$>(3\text{-}5)*10^3$ cuboidal GC	$>19*10^6$ cuboidal GC	$>94*10^6$ cuboidal GC	$>47*10^6$ cuboidal GC	–
	FSHR	–	–	–	+	+	+	+++	++++
	Hormone production	–	–	–	–	–	E_2	E_2	P, E_2
Theca cell	LHR	–	–	–	+	+	+	+++	++++
	Hormone production	–	–	–	P	P, ASD	P, ASD	P	ASD

pregnenolone to androgens in the cal cells. Later the androgens diffuse to the granulosa cells and are transformed into estrogens under the catalysis of cytochrome P450 aromatase, which is stimulated by FSH [29, 30].

Researches have indicated that EDCs can inhibit ovarian steroidogenesis. For example, neonatal female caiman exposed to E2 or BPA presents higher estrogen serum levels [11]. DES, BP, and OP reduce estradiol and testosterone levels, and GEN causes a decline in testosterone levels and cAMP by stimulating the activity of aromatase enzyme [31]. DEHP inhibits the production of progesterone, androstenedione, testosterone, and estradiol from antral follicles, by inhibiting the expression of side-chain cleavage related enzymes such as 17α-hydroxylase-17,20-desmolase, 17β-hydroxysteroid dehydrogenase, and aromatase [13]. Cytochrome P450 aromatase is the critical steroidogenic enzyme that is responsible for the conversion of androgens to estrogens. EDCs disrupt steroidogenesis by interfering with the gene expression of cytochrome P450 aromatase. Varieties of EDCs have been proved to potentially disturb reproductive function by dysregulating the expression of CYP19 genes through differential transcriptional modulation [32, 33]. Zebrafishes exposed to an estrogenic mixture of 11 EDCs can be observed an alteration of CYP19A1 activity and Mtf-1 and tfap2c transcription factor [34]. Furthermore, Benzo[α] pyrene (B[α]P) alters the expression of CYP2N23. BPA changes CYP2P18, CYP2P19, and 4-OP and disturbs CYP2AD12 [35]. DES and tetrabromobisphenol A suppress CYP17 but not CYP19 activity, indicating different mechanisms of different EDCs acting on these cytochrome p450 aromatase [36].

EDCs not only can alter the enzymatic activity of cytochrome p450 aromatase, but also can interfere with the gonadotropin receptor signaling second messengers such as cAMP or modulate the Ca^{2+} associated metabolic pathway. For example, 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), a metabolite of DDT, suppresses progesterone synthesis through inhibiting the generation of gonadotropin receptor signaling second messengers cAMP [37], and also decreases the gene expression of P450 cholesterol side-chain cleavage (P450scc) [38]. Furthermore, Younglai et al. [39] have found elevations of [Ca(2+)](cyt) in granulosa-lutein cells, suggesting DDT can also modulate $Ca2^{+}$-dependent pathways.

3.2.3 Nuclear Receptor Signaling

There are a variety of mechanisms in which EDCs exert harmful effects, one of which is attributed to the interaction with nuclear hormone receptors (NHRs). EDCs interfere with genomic and non-genomic estrogen receptor (ER) activity via directly binding with two ERs (ERα and ERβ), by the assistance of transcription factors like the aryl hydrocarbon receptor (AhR) or through modulation of critical enzymes during estrogen synthesis or metabolism [40]. Estrogenic signal networks are divided into the intracellular and the extracellular pathways. The intracellular pathways include the genomic pathway and the non-genomic pathway, involving in

the transcription of target genes and signal transductions via binding with membrane receptors, respectively. The extracellular pathways involve other modulating factors such as other growth factors, cytokines, and hormones [40, 41]. Not only the estrogenic or androgenic receptors, multiple studies have indicated that activity of the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), the thyroid hormone receptors (TRs), the retinoid X receptors (RXRs), the estrogen related receptors (ERRs), or the peroxisome proliferator-activated receptors (PPARs) could also be affected by EDCs, reviewed by Albane et al. [42].

Different kinds of EDCs exhibit different ER responsive properties. For example, kaempferol, coumestrol, daidzein, and genistein can mediate both ERα and ERβ ERE-mediated activities, while 2-2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane, bisphenol AF, and BPA activate the ERα pathway. Besides, only a few kinds of EDCs activate the tethered mechanism through ERα or ERβ [43]. In addition, Sheikh et al. [44] have elucidated that BPA, MBP (4-Methyl-2, 4-bis (4-hydroxyphenyl) pent-1-ene), 4-tert-OP, and 4-NP exhibited high binding affinity with sex hormone-binding globulin (SHBG), indicating that these EDCs can potentially interfere or disrupt the steroid-binding function.

3.2.4 Epigenetic Modification

As we have mentioned above, the reproductive system is sensitive to EDCs, especially during the early critical development window, which may lead to subtle epigenetic alteration and thus cause permanent or even multigenerational or transgenerational changes [45–48]. The epigenetic modification of the germline includes histone modifications, DNA methylation, non-coding RNAs, or alterations in chromatin structure [49, 50]. For example, prenatal exposure to the environmental doses of phthalate mixture induced multigenerational and transgenerational effects on female reproduction [51]. Brehm et al. [52] have suggested that prenatal DEHP exposure (750 mg/kg/d) can alter estrous cyclicity, augment estradiol levels (F1 and F3), increase the presence of ovarian cysts (F1), decreased testosterone levels and folliculogenesis (F1, F2, and F3), reduce progesterone levels (F2), decrease inhibin B levels (F1 and F3), and change gonadotropin hormone levels (F1 and F3). We will discuss it further in the later section.

3.3 BPA Reduces Fertility Ability, Reduces the Primordial Follicle Pool Reserve, Leads to Premature Ovarian Failure, Disturbs the Estrous Cycle, and Disrupts Steroidogenesis in Different Animal and Human Models

In this section, we will focus on BPA, which is a plasticizer used commonly and widely in food and drink containers, plastic products, epoxy resins [53], and dental materials [54]. BPA can leach out from several products in the condition of high temperatures, acidic or alkaline environment, UV rays, or repeated use [55]. BPA can be detected in plasma, serum, sweat, urine, breast milk, placental tissue, umbilical cords, amniotic fluid, and fetal serum [56]. More importantly, BPA is considered a reproductive toxicant because it can be detected in various reproductive tissues [57]. Controversially, however, Teeguarden et al. [58] summarized methods of serum BPA measurement but they found that BPA serum concentrations in humans were unmeasurable, and contributed limited or no estrogenicity.

According to the World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO), the human exposure levels to BPA are estimated to be 0.4 ~ 1.4 mg/kg bw/d [59]. Although the safe reference dose of BPA proposed by the US Environmental Protection Agency is 50 mg/kg bw/d [60], previous studies have indicated that BPA has potentially negative impacts even at much lower doses in a non-monotonic dose-response manner [61–63].

A study has revealed that BPA can be measured in the urine of almost all the women undergoing IVF treatment, of which the concentrations are negatively associated with the ovarian response, including peak estradiol levels and the number of oocytes retrieved [64]. Besides, the plasma concentrations of BPA are transient with short half-lives (< 2 h) in pregnant women, while sustained in fetal plasma [65]. Furthermore, BPA concentrations in serum of women carrying fetuses with abnormal karyotypes in the early second trimester are higher than the control group [66], which raises concerns about the adverse impacts of BPA on human development.

Human and animal studies have illustrated that BPA exposure will reduce fertility ability by disrupting the primordial follicle pool, interfering with oogenesis, and disturbing ovary functions such as steroidogenesis and the estrous cycle. Worse still, BPA was proved to have transgenic effects that may have impacts on offspring.

Researches on infertility women have revealed that higher urinary BPA concentrations are correlated with lower antral follicle counts [67]. In animal models, scientists have obtained consistent results. In rat models, BPA exposure (2.5 and 250 mg/kg bw/d) at PND 21 can reduce the numbers of primordial, primary, preantral, and total healthy follicle numbers [55]. Atretic follicles, cysts formation, and separation of granulosa cells can be observed in rats' ovaries after exposure to BPA orally for 4 weeks [68]. Perinatal exposure to BPA by the oral route during gestation and breastfeeding can decrease recruitment of primordial follicles, increase the number of corpora lutea, and ovulate oocytes in rats ovaries [69]. Neonatal rats subcutaneous exposed to BPA (1 mg/kg) exhibits multiple cystic follicles and

decreased area of corpora lutea (CL) in the ovary [70]. In mice models, BPA can facilitate the transition from primordial to primary follicles, accelerate the dissipation of the primordial follicle pool, and inhibit meiosis I by abnormal spindle assembly [71]. BPA (10 or 100 μM) exposure to neonatal mice isolated ovaries significantly reduces germ nest breakdown and primordial follicle assembly [72]. Besides, BPA (30 M) exposure to in vitro mice oocytes during follicular development shows the reduction of granulosa cell proliferation and total estrogen production, but it still can develop and form antral-like cavities [73]. In lambs models, prenatal low dose BPA (50 μg/kg/day) exposure affects the ovarian follicular dynamics by reducing primordial follicle pool reserve and stimulating follicular recruitment and development [74]. Veiga-Lopez et al. [75] have also proved that prenatal BPA treatment causes ovarian follicular dynamics disruption on sheep. BPA exposure can shorten the time interval between the estradiol rise and the preovulatory LH surge, and disturb follicular count trajectories. Besides, BPA early exposure results in the augmentation of the number of multi-oocyte follicles (MOFs), granulosa/theca cells in antral follicles as well as antral atretic follicles, because folliculogenesis acceleration increases the incidence of atretic follicles [74]. What's more, Gieske et al. [76] have also suggested that prenatal and postnatal exposure to BPA results in increased formation of MOFs and antral follicles in the primate model. One of the underlying mechanisms is that BPA disrupts the follicular progression. BPA induces follicular atresia via disrupting the follicular progress by interfering with the previtellogenic and vitellogenic phases. BPA exposure may stimulate follicular recruitment of the primary follicular recruitment on the primary stage and then forces the follicular transition from stage III to IV with enlarged stage IV follicles, thereby inducing atresia [77]. What's more, BPA affects follicle numbers and constituent via interfering with the follicle development-related genes such as down-regulation of the expression of oocyte-specific histone H1 variant (H1FOO) and factor in the germline alpha (FIGLA) genes, and up-regulation of anti-Mullerian hormone (AMH) genes expression [78]. In addition, BPA may impair folliculogenesis by increasing the expression of oocytes specific genes such as Sohlh2 (spermatogenesis and oogenesis helix-loop-helix), Nobox (newborn ovary homeobox), Lhx8 (LIM homeobox 8), and FIGLA. BPA disturbs the normal process of folliculogenesis by blocking the demethylation of CpG sites of the Lhx8 gene in oocytes [72]. Furthermore, postnatal exposure to BPA can dose-dependently disturb the early ovary development by disrupting the Notch signaling pathway [79].

BPA orally exposure to young adult mice (50 μg/kg bw/day) has been shown to reduce the fertilization ability of oocytes rather than affect ovulation [80]. Acute low doses exposure of BPA (3, 5 mg/L) to zebrafish can disrupt oogenesis, displaying severe deterioration of ovarian tissue with distorted and immature oocytes, and the increased number of atretic oocytes [81]. Persistent unenclosed oocytes in the medullary region and small non-growing oocytes in secondary and antral follicles have been presented in rhesus monkeys when continuously exposed to BPA before birth [82]. Consistently, BPA exposure to human oocytes shows decreased oocyte survival, increased oocyte degeneration, and increased MLH1 (crossover marker) foci number, which indicates BPA can act as a toxic substance and affect meiotic

prophase such as pairing-synapsis and recombination processes, as well as decrease oocyte survival [83]. Numerous previous studies have attempted to explain the underlying mechanisms of the impacts of BPA on oocyte development. Researches have suggested there is lipid droplet accumulation, chromatin condensation in the nuclei of granulosa cells, and autophagosomes in rats' ovaries at 4 weeks post-exposure to BPA [68]. Exposure to BPA during early gestation age may have adverse impacts on meiosis, thus disrupts the development of the oocytes. For example, low dose BPA exposures during mid-gestation lead to oocytes displaying gross aberrations in the meiotic prophase, including enhanced levels of recombination and synaptic defects, and an increase in aneuploid eggs and embryos in rats [84]. In isolated mice ovaries model, BPA exposure (30 M) has adverse effects on the meiotic spindle, thereby hindering meiosis progression [73]. Besides, BPA exposures to rhesus monkeys during middle and gestational age can induce chromosome segregation disturbances and MOFs increase, respectively [82]. Also, BPA has been proven to have genotoxic and cytogenetic, but not mutagenic effects. BPA can interfere with the gene expressions related to meiosis. Prenatal exposure to low dose BPA may have impacts on early oogenesis by disturbing the gene expression, especially that correlated with the onset of meiosis [85]. BPA exposure to mice results in more oocytes in germ cell cyst and less primordial follicle counts through inhibiting the meiotic progression of oocytes, via down-regulated mRNA expression of specific meiotic genes, including Dmc1, Stra8, Scp3, and Rec8 [86]. BPA exposure to in vitro embryonic stem (ES) cells significantly upregulates the expression of the meiotic entry gene Stra8, accompanied by aggregated Sycp3 signal localized in nuclei and up-regulation of ovarian markers (Foxl2 and Wnt4), which can help to explain how BPA affects germ cell differentiation [87]. Furthermore, BPA can deteriorate egg quality through decreasing HDAC7 expression in mice ovary and eggs, while increasing H3K9 and H4K16 acetylation [88]. Besides, BPA exposure to ovaries induces a significant elevation in micronucleus frequency, and conventional chromosome aberrations such as breaks, gaps, and fragments increased [89]. Furthermore, BPA exposure leads to chromosome synapsis impairment and disturbance of meiotic double-strand break repair (DSBR) progression, which is essential to genomic integrity maintenance during meiosis [90]. Oocytes exposed to BPA show a significant increment of Rpa, Spo11, H2ax, and Blm genes involved in DSB generation, signaling, and repair, as well as up-regulation of Erα, Erβ, and Errγ genes related to estrogen receptor [91]. Ganesan et al. [92] have demonstrated that BPA can induce ovarian DNA damage, with significant increased DNA DSB marker cH2AX and ATM before follicle loss. Besides, they have observed ovary that may activate DNA repairment and xenobiotic biotransformation to protect oocyte from damage, or activate cell death signaling to deplete follicles.

Previous researches have indicated that BPA adversely affects ovarian functions, including disturbing estrous cycle and steroidogenesis. Neonatal period exposure to BPA causes advanced puberty onset [70] and irregular estrous cycle [70]. Prepubertal exposure to BPA in mice will present advanced puberty onset [93, 94], ovary weight reduction [78, 93, 74], disturb estrous cycle and duration [93, 94], lower E2 response during in vitro fertilization (IVF) [95], and diminish ovarian reserve [96]. Exposure

to BPA during the implantation period may have potential effects on adverse pregnancy outcomes and reduction of litter size or implantation rate [97].

In vitro experiments in isolated porcine granulosa cells have illustrated that BPA exposure can disturb steroidogenesis with the progesterone level decreased [98]. However, BPA exhibits non-monotonic dose effects on ovarian steroidogenesis, which may be attributed to different alteration properties of steroidogenic enzymes. For instance, steroidogenic gene expressions are promoted by BPA at lower concentrations (5 and 15 μg/L) while inhibited at higher concentrations (50 μg/L) [99]. The basal progesterone level elevates when BPA is at 10^{-8} M to 10^{-5} M, and FSH-stimulated progesterone level is promoted when BPA is at 10^{-7} M and 10^{-6} M. In comparison, BPA at 10^{-4} M inhibits the basal and gonadotropin-stimulated progesterone production [100]. Besides, BPA increases progesterone levels and elevates mRNA expression of steroidogenic acute regulatory protein (StAR) and P450scc at 10^{-7} to 10^{-5} M. In contrast, progesterone levels and P450scc expression are decreased, and StAR expression is increased at 10^{-4} M. BPA exposure at the concentrations of 10^{-6} to 10^{-4} M inhibits estradiol levels and P450 aromatase expression in a dose-dependent manner [101]. BPA exposure (10^{-7} to 10^{-4} M) shows enhancive testosterone synthesis, augmentation of mRNA expression of cholesterol side-chain cleavage enzyme (P450scc), 17- hydroxylase (P450c17), and StAR in rat ovarian theca-interstitial cells. BPA (20 mg/ml) disturbs progesterone and estradiol synthesis via down-regulated gene expression of 3b-hydroxysteroid dehydrogenase (3b-HSD), cytochrome P450 side-chain cleavage (CYP11A1) and CYP19A1 related to encode steroidogenesis enzymes [102]. In the studies of isolated human luteinized granulosa cells, BPA exposure (10, 100 μg/mL) lessens the expression of steroidogenic enzyme Cyp11A1 and StAR in mice antral follicles in vitro, causing a reduction of steroidogenesis including progesterone, androstenedione, testosterone, and estradiol. However, these effects can be reversed by the removal of BPA in acute exposure [103]. Perinatal rats exposed to BPA (0.5, 50 μg/kg day) exhibit higher levels of mRNA expression of 3-β-hydroxysteroid dehydrogenase and serum progesterone, and lower levels of androgen receptor (AR) [69]. Banerjee et al. have elucidated that catalase mediated reproductive damage to granulosa cells in rats after BPA exposure. BPA exposure results in the elevation of nitric oxide, lipid peroxidation, pro-inflammatory cytokine, serum FSH and LH levels, as well as reduction of the catalase expression and estrogen or progesterone levels, of which the effects can be augmented by pretreatment with catalase blocker [104]. Zhang et al. have also revealed that BPA action may involve epigenetic regulation, as well as ER and AR signaling, nuclear receptor subfamily 5, group A, number 1 (Nr5a1) pathway [99].

We have reviewed that BPA exposure has effects on follicular formation, oocyte development, and steroidogenesis based on human and animal studies. Various studies have also observed that BPA has transgenerational effects on steroidogenesis and folliculogenesis. In utero low doses of BPA exposure will affect early ovarian development and reduce the fecundity of females in the subsequent generations.

BPA exposure (20 μg/L) to zebrafish can diminish female adult fertility up to F2 [105]. A further study [106] has shown that in utero BPA exposure not only affects

F1 but also reduces fecundity on the subsequent three generations. Wang et al. [107] have indicated that F1 female mice exposed to low doses of BPA exhibit various fertility problems, significantly increased dead pups and estrus cycle disturbance. In vivo [107] and in vitro [108] studies have reached a consistent result that BPA exposure may inhibit germ cell nest breakdown via altering the expression of critical ovarian apoptotic genes, such as decreasing expression of pro-apoptotic factors and increasing expression of anti-apoptotic factors. Shi et al. [94] also have found transgenerational effects of BPA on the earlier onset of puberty, estrous cyclicity disturbance, fertility problem, serum testosterone elevation, and primary and secondary follicle counts reduction. Early BPA exposure to F1 might reduce the relative ovary weight in F2. However, they did not observe that BPA exposure affected germ cell nest breakdown, primordial, primary, or secondary follicles in F3 ovaries on PND 4, whereas exposure to BP tended to increase germ cells in nests. Likewise, Berger et al. [109] have shown no transgenerational effects on germ cell nest breakdown and gene expression on PND 4. Collectively, these data have suggested that BPA directly targets the ovary to inhibit germ cell nest breakdown in the F1 generation, but not the subsequent generations.

One of the reasons to explain BPA exposure affecting female germ cells is that BPA may change the gene expression pattern. Liu et al. [110] have elucidated that BPA exposure to zebrafish can result in the global DNA demethylation in the ovary via altering transcripts of DNA methylation/demethylation-associated genes: glycine N-methyltransferase (GNMT), DNA methyltransferase (DNMTs), and ten-eleven translocation. The global DNA methylation level is significantly elevated in the ovary, which can be affected by DNMTs expression alteration [111]. BPA exposure can affect the DNA methylation of imprinting genes by decreasing gene expressions of Igf2r, Peg3, and H19, and curtailing mRNA expressions of specific meiotic genes, as well as increasing Nobox mRNA expression in fetal mouse germ cells [112]. Chao et al. [71] have illustrated that BPA exposure to CD-1 mice results in reduced imprinting gene expressions of Igf2r and Peg3 via the ER signaling pathway during oogenesis. A study conducted on zebrafish has demonstrated that exposure to 5 μg/L BPA may promote apoptosis in mature follicles and downregulate oocyte maturation-promoting signals, probably via alterations in the chromatin structure mediated by histone modifications [113]. Collectively, these data have indicated that the detrimental impacts of BPA on the female reproductive system may be due to the deregulation of epigenetic mechanisms.

In addition to the transgenic effects on germ cell development, BPA can also affect the sex hormone production on subsequent generations. In utero, BPA exposure will reduce cytochrome P450 aromatase mRNA levels, estradiol levels, and preantral follicle numbers in the F1 generation. On the other hand, it may decrease testosterone levels and alter mRNA expression of cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 aromatase, 3β-hydroxysteroid dehydrogenase 1, and steroidogenic acute regulatory protein in the F2 generation [114]. Moustafa et al. [115] have illustrated that exposure to BPA (50 and 200 mg/kg), especially at 200 mg/kg, results in a clear marked DNA fragmentation and an increase in ER expression in the ovary, as well as serum estrogen elevation of both dam and F1

female rats. The methylation level of ovarian cytochrome P450 aromatase gene (CYP19A1A) is drastically reduced and increased, respectively, by 7- and 35-day BPA exposure. CYP19A1A mRNA expression in the ovary is reversely correlated to methylation levels of the four CpGs at the 5-flanking region [111]. These results suggest that the alteration of CYP19A1A expression can be related to the modification of DNA methylation status.

Although we have presented the human and animal data of the pernicious effects of BPA, the experiment doses of BPA are higher than the environmental doses. One study [116] has illustrated that there was no significant alteration in transcriptome and ovarian morphology in sheep ovaries when in vitro exposure to BPA at environmental doses (10^{-7} M and 10^{-8} M). Therefore, we are not sure about the exact doses of BPA which can exert its harmful effects. Further studies to prove different dose effects of BPA are needed.

3.4 The Future Direction of Prevention and Solution Methods

Due to the adverse health impacts caused by BPA exposure, BPA has begun to be eliminated from various consumer products or be replaced by substitutes such as bisphenol S (BPS), which is structurally similar to BPA. However, the structural similarity implicates that BPS may have analogical adverse effects. For instance, neonatal exposure to BPS (5 and 50 mg/kg) causes BPA like endocrine and structural changes in female rats [117]. Prenatal BPS exposure alters the expression of estrogen-responsive genes in both the uterus and ovary, displaying heightened responses in the uterus and diminished responses in the ovary, respectively [118]. Besides, BPS exposure can accelerate ovarian follicular development in prepubertal female mice. BPS causes significant germline apoptosis and embryonic lethality in the genetic model system Caenorhabditis elegans [119]. BPS administration in low or high doses can lead to female reproductive toxicities and oxidative stress in mice [120]. In consequence, these findings urge more researches and safe novel alternatives to BPA.

In addition to looking for new alternatives, studies also attempt to discover new therapies that can reverse the harmful effects of BPA. For example, Tualang honey has a protective effect on minimizing BPA-induced ovarian toxicity by modifying the estrous cycle and reducing numbers of atretic follicles [121]. Further study suggests that Tualang honey can change the ERα, ERβ, and C3 expressions and distribution in BPA-treated rats [122]. High doses of BPA (100 mg/kg/day) were pernicious to ovaries, and vitamins may have protective effects [123]. Vitamin C can be a potential antidote in a condition of ovarian toxification by BPA exposure [124]. 1,25-dihydroxyvitamin D3 (1,25D3) may reverse the detrimental effect of BPA by increasing mtDNA content, attenuating mtDNA deletion, inhibiting reduction in E2 secretion and COXI expression. Besides, 1,25D3 can increase

mitochondrial biogenesis-related proteins by PI3K-Akt signaling and elevate cellular oxygen consumption rate and ATP production [125]. What's more, Ficus deltoidea may protect against BPA-induced toxicity of the pituitary-ovarian axis in prepubertal female rats. It can restore normal estrous cycle, normalize FSH and progesterone levels, as well as reduce the number of atretic follicles [126].

Because humans are exposed to low doses and mixtures of various kinds of EDCs at different life stages, exploration of the exact effects of EDCs on human life turns to be complicated. This means that rather than the investigation of single-exposure, dose-response effects of pure chemical, we need new strategies to conduct a risk assessment on mixtures of daily EDCs such as the safe exposure threshold of mixtures rather than single pure compounds. Besides, EDCs are ubiquitous in the environment hence humans in different life stages from embryo, fetus, infant, child to adolescence, adulthood, and aging are vulnerable to EDCs. Therefore, researches on EDCs should not only focus on the dose effects, but also the timing effects. In conclusion, EDCs have been proven to be of significant adverse effects on human life. In order to diminish even reverse the harmful impacts of EDCs, we still need further researches.

References

1. Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek N, Soto A, Woodruff T, Vom Saal F. Endocrine-disrupting chemicals and public health protection: a statement of principles from the Endocrine Society. Endocrinology. 2012;153(9):4097–110.
2. Bergman Å, Heindel JJ, Jobling S, Kidd K, Zoeller TR, Organization WH. State of the science of endocrine disrupting chemicals 2012. Geneva, Switzerland: World Health Organization; 2013.
3. Gore AC, Crews D, Doan LL, La Merrill M, Patisaul H, Zota A. Introduction to endocrine disrupting chemicals (EDCs). A guide for public interest organizations and policy-makers. Washington, DC: Endocrine Society; 2014.
4. Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev. 2009;30(4):293–342.
5. Olsén L, Lampa E, Birkholz DA, Lind L, Lind PM. Circulating levels of bisphenol A (BPA) and phthalates in an elderly population in Sweden, based on the prospective investigation of the vasculature in Uppsala seniors (PIVUS). Ecotoxicol Environ Saf. 2012;75:242–8.
6. Casas L, Fernández MF, Llop S, Guxens M, Ballester F, Olea N, Irurzun MB, Rodríguez LSM, Riaño I, Tardón A. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int. 2011;37(5):858–66.
7. Zhu Y, Huang B, Li QX, Wang J. Organochlorine pesticides in follicular fluid of women undergoing assisted reproductive technologies from Central China. Environ Pollut. 2015;207:266–72.
8. Gerona RR, Woodruff TJ, Dickenson CA, Pan J, Schwartz JM, Sen S, Friesen MW, Fujimoto VY, Hunt PA. Bisphenol-A (BPA), BPA glucuronide, and BPA sulfate in midgestation umbilical cord serum in a northern and Central California population. Environ Sci Technol. 2013;47(21):12477–85.
9. Golovleva L, Polyakova A, Pertsova R, Finkelshtein Z. The fate of methoxychlor in soils and transformation by soil microorganisms. J Environ Sci Health Part B. 1984;19(6):523–38.

10. Johansson HKL, Jacobsen PR, Hass U, Svingen T, Vinggaard AM, Isling LK, Axelstad M, Christiansen S, Boberg J. Perinatal exposure to mixtures of endocrine disrupting chemicals reduces female rat follicle reserves and accelerates reproductive aging. Reprod Toxicol. 2016;61:186–94. https://doi.org/10.1016/j.reprotox.2016.03.045.
11. Stoker C, Beldoménico PM, Bosquiazzo VL, Zayas MA, Rey F, Rodríguez H, Muñoz-de-Toro M, Luque EH. Developmental exposure to endocrine disruptor chemicals alters follicular dynamics and steroid levels in Caiman latirostris. Gen Comp Endocrinol. 2008;156(3):603–12. https://doi.org/10.1016/j.ygcen.2008.02.011.
12. Gupta RK, Singh JM, Leslie TC, Meachum S, Flaws JA, Yao HHC. Di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate inhibit growth and reduce estradiol levels of antral follicles in vitro. Toxicol Appl Pharmacol. 2010;242(2):224–30. https://doi.org/10.1016/j.taap.2009.10.011.
13. Hannon PR, Brannick KE, Wang W, Gupta RK, Flaws JA. Di(2-ethylhexyl) phthalate inhibits antral follicle growth, induces atresia, and inhibits steroid hormone production in cultured mouse antral follicles. Toxicol Appl Pharmacol. 2015;284(1):42–53. https://doi.org/10.1016/j.taap.2015.02.010.
14. Cevasco A, Urbatzka R, Bottero S, Massari A, Pedemonte F, Kloas W, Mandich A. Endocrine disrupting chemicals (EDC) with (anti)estrogenic and (anti)androgenic modes of action affecting reproductive biology of Xenopus laevis: II. Effects on gonad histomorphology. Comp Biochem Physiol Part C: Toxicol Pharmacol. 2008;147(2):241–51. https://doi.org/10.1016/j.cbpc.2007.10.001.
15. Mlynarčíková A, Nagyová E, Ficková M, Scsuková S. Effects of selected endocrine disruptors on meiotic maturation, cumulus expansion, synthesis of hyaluronan and progesterone by porcine oocyte–cumulus complexes. Toxicol In Vitro. 2009;23(3):371–7.
16. Nikaido Y, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A. Effects of Prepubertal exposure to Xenoestrogen on development of estrogen target organs in female CD-1 mice. In Vivo. 2005;19(3):487–94.
17. Qiao L, Zheng L-X, Cai D. Study on the levels of the bisphenol A, octylphenol, 4-nonylphenol in serum of precocious girls. J Hygiene Res. 2010;39(1):9–12.
18. Lea RG, Amezaga MR, Loup B, Mandon-Pépin B, Stefansdottir A, Filis P, Kyle C, Zhang Z, Allen C, Purdie L, Jouneau L, Cotinot C, Rhind SM, Sinclair KD, Fowler PA. The fetal ovary exhibits temporal sensitivity to a 'real-life' mixture of environmental chemicals. Sci Rep. 2016;6:22279. https://doi.org/10.1038/srep22279.
19. Zama AM, Uzumcu M. Fetal and neonatal exposure to the endocrine disruptor Methoxychlor causes epigenetic alterations in adult ovarian genes. Endocrinology. 2009;150(10):4681–91. https://doi.org/10.1210/en.2009-0499.
20. Zama AM, Uzumcu M. Targeted genome-wide methylation and gene expression analyses reveal signaling pathways involved in ovarian dysfunction after developmental EDC exposure in Rats1. Biol Reprod. 2013;88(2):1–13. https://doi.org/10.1095/biolreprod.112.104802.
21. Heindel JJ, Balbus J, Birnbaum L, Brune-Drisse MN, Grandjean P, Gray K, Landrigan PJ, Sly PD, Suk W, Slechta DC. Developmental origins of health and disease: integrating environmental influences. Endocrinology. 2015;156(10):3416–21.
22. Saeidnia S, Abdollahi M. Toxicological and pharmacological concerns on oxidative stress and related diseases. Toxicol Appl Pharmacol. 2013;273(3):442–55. https://doi.org/10.1016/j.taap.2013.09.031.
23. Luderer U. Chapter four - ovarian toxicity from reactive oxygen species. In: Litwack G, editor. Vitamins & hormones, vol. 94. Cambridge, MA: Academic Press; 2014. p. 99–127. https://doi.org/10.1016/B978-0-12-800095-3.00004-3.
24. Wang W, Craig ZR, Basavarajappa MS, Gupta RK, Flaws JA. Di (2-ethylhexyl) phthalate inhibits growth of mouse ovarian antral follicles through an oxidative stress pathway. Toxicol Appl Pharmacol. 2012;258(2):288–95. https://doi.org/10.1016/j.taap.2011.11.008.

25. Gupta RK, Miller KP, Babus JK, Flaws JA. Methoxychlor inhibits growth and induces atresia of Antral follicles through an oxidative stress pathway. Toxicol Sci. 2006;93(2):382–9. https://doi.org/10.1093/toxsci/kfl052.
26. Sobinoff AP, Pye V, Nixon B, Roman SD, McLaughlin EA. Adding insult to injury: effects of xenobiotic-induced Preantral Ovotoxicity on ovarian development and oocyte fusibility. Toxicol Sci. 2010;118(2):653–66. https://doi.org/10.1093/toxsci/kfq272.
27. Armstrong D, Goff A, Dorrington J. Ovarian follicular development and function. New York: Raven Press; 1979. p. 169–82.
28. Hillier SG, Whitelaw PF, Smyth CD. Follicular oestrogen synthesis: the 'two-cell, two-gonadotrophin' model revisited. Mol Cell Endocrinol. 1994;100(1-2):51–4.
29. Erickson GF, Magoffin DA, Dyer CA, Hofeditz C. The ovarian androgen producing cells: a review of structure/function relationships. Endocr Rev. 1985;6(3):371–99.
30. Richards JS. Hormonal control of gene expression in the ovary. Endocr Rev. 1994;15(6):725–51.
31. Myllymäki S, Haavisto T, Vainio M, Toppari J, Paranko J. In vitro effects of diethylstilbestrol, genistein, 4-tert-butylphenol, and 4-tert-octylphenol on steroidogenic activity of isolated immature rat ovarian follicles. Toxicol Appl Pharmacol. 2005;204(1):69–80.
32. Kazeto Y, Place AR, Trant JM. Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (Danio rerio) juveniles. Aquat Toxicol. 2004;69(1):25–34. https://doi.org/10.1016/j.aquatox.2004.04.008.
33. Wang J, Liu X, Wang H, Wu T, Hu X, Qin F, Wang Z. Expression of two cytochrome P450 aromatase genes is regulated by endocrine disrupting chemicals in rare minnow Gobiocypris rarus juveniles. Comp Biochem Physiol Part C: Toxicol Pharmacol. 2010;152(3):313–20.
34. Urbatzka R, Rocha E, Reis B, Cruzeiro C, Monteiro RAF, Rocha MJ. Effects of ethinylestradiol and of an environmentally relevant mixture of xenoestrogens on steroidogenic gene expression and specific transcription factors in zebrafish. Environ Pollut. 2012;164:28–35. https://doi.org/10.1016/j.envpol.2012.01.018.
35. Puthumana J, Kim B-M, Jeong C-B, Kim D-H, Kang H-M, Jung J-H, Kim I-C, Hwang U-K, Lee J-S. Nine co-localized cytochrome P450 genes of the CYP2N, CYP2AD, and CYP2P gene families in the mangrove killifish Kryptolebias marmoratus genome: identification and expression in response to B [α] P, BPA, OP, and NP. Aquat Toxicol. 2017;187:132–40.
36. Roelofs MJE, Piersma AH, van den Berg M, van Duursen MBM. The relevance of chemical interactions with CYP17 enzyme activity: assessment using a novel in vitro assay. Toxicol Appl Pharmacol. 2013;268(3):309–17. https://doi.org/10.1016/j.taap.2013.01.033.
37. Jorge Chedrese P, Feyles F. The diverse mechanism of action of dichlorodiphenyldichloroethylene (DDE) and methoxychlor in ovarian cells in vitro. Reprod Toxicol. 2001;15(6):693–8. https://doi.org/10.1016/S0890-6238(01)00172-1.
38. Crellin N, Kang H, Swan C, Chedrese P. Inhibition of basal and stimulated progesterone synthesis by dichlorodiphenyldichloroethylene and methoxychlor in a stable pig granulosa cell line. Reproduction. 2001;121(3):485–92.
39. Younglai E, Kwan T, Kwan C-Y, Lobb D, Foster W. Dichlorodiphenylchloroethylene elevates cytosolic calcium concentrations and oscillations in primary cultures of human granulosa-lutein cells. Biol Reprod. 2004;70(6):1693–700.
40. Shanle EK, Xu W. Endocrine disrupting chemicals targeting estrogen receptor signaling: identification and mechanisms of action. Chem Res Toxicol. 2011;24(1):6–19. https://doi.org/10.1021/tx100231n.
41. Kiyama R, Wada-Kiyama Y. Estrogenic endocrine disruptors: molecular mechanisms of action. Environ Int. 2015;83:11–40. https://doi.org/10.1016/j.envint.2015.05.012.
42. le Maire A, Bourguet W, Balaguer P. A structural view of nuclear hormone receptor: endocrine disruptor interactions. Cell Mol Life Sci. 2010;67(8):1219–37. https://doi.org/10.1007/s00018-009-0249-2.
43. Li Y, Luh CJ, Burns KA, Arao Y, Jiang Z, Teng CT, Tice RR, Korach KS. Endocrine-disrupting chemicals (EDCs): in vitro mechanism of estrogenic activation and differential

effects on ER target genes. Environ Health Perspect. 2013;121(4):459–66. https://doi.org/10.1289/ehp.1205951.
44. Sheikh IA, Tayubi IA, Ahmad E, Ganaie MA, Bajouh OS, AlBasri SF, Abdulkarim IMJ, Beg MA. Computational insights into the molecular interactions of environmental xenoestrogens 4-tert-octylphenol, 4-nonylphenol, bisphenol A (BPA), and BPA metabolite, 4-methyl-2, 4-bis (4-hydroxyphenyl) pent-1-ene (MBP) with human sex hormone-binding globulin. Ecotoxicol Environ Saf. 2017;135:284–91. https://doi.org/10.1016/j.ecoenv.2016.10.005.
45. Heindel JJ, Vandenberg LN. Developmental origins of health and disease: a paradigm for understanding disease cause and prevention. Curr Opin Pediatr. 2015;27(2):248–53. https://doi.org/10.1097/MOP.0000000000000191.
46. Rosenfeld CS. The epigenome and developmental origins of health and disease. Cambridge, MA: Academic Press; 2015.
47. Hoffman DJ, Reynolds RM, Hardy DB. Developmental origins of health and disease: current knowledge and potential mechanisms. Nutr Rev. 2017;75(12):951–70.
48. Rattan S, Flaws JA. The epigenetic impacts of endocrine disruptors on female reproduction across generations. Biol Reprod. 2019;101(3):635–44.
49. Xin F, Susiarjo M, Bartolomei MS. Multigenerational and transgenerational effects of endocrine disrupting chemicals: a role for altered epigenetic regulation? Semin Cell Dev Biol. 2015;43:66–75. https://doi.org/10.1016/j.semcdb.2015.05.008.
50. Skinner MK. Endocrine disruptors in 2015: epigenetic transgenerational inheritance. Nat Rev Endocrinol. 2016;12(2):68–70. https://doi.org/10.1038/nrendo.2015.206.
51. Zhou C, Gao L, Flaws JA. Exposure to an environmentally relevant phthalate mixture causes Transgenerational effects on female reproduction in mice. Endocrinology. 2017;158(6):1739–54. https://doi.org/10.1210/en.2017-00100.
52. Brehm E, Rattan S, Gao L, Flaws JA. Prenatal exposure to Di(2-Ethylhexyl) phthalate causes long-term Transgenerational effects on female reproduction in mice. Endocrinology. 2018;159(2):795–809. https://doi.org/10.1210/en.2017-03004.
53. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139–77.
54. Fleisch AF, Sheffield PE, Chinn C, Edelstein BL, Landrigan PJ. Bisphenol A and related compounds in dental materials. Pediatrics. 2010;126(4):760–8. https://doi.org/10.1542/peds.2009-2693.
55. Patel S, Brehm E, Gao L, Rattan S, Ziv-Gal A, Flaws JA. Bisphenol A exposure, ovarian follicle numbers, and female sex steroid hormone levels: results from a CLARITY-BPA study. Endocrinology. 2017;158(6):1727–38.
56. Patel S, Zhou C, Rattan S, Flaws JA. Effects of endocrine-disrupting chemicals on the ovary. Biol Reprod. 2015;93(1):21–9.
57. Ziv-Gal A, Flaws JA. Evidence for bisphenol A-induced female infertility: a review (2007–2016). Fertil Steril. 2016;106(4):827–56.
58. Teeguarden J, Hanson-Drury S, Fisher JW, Doerge DR. Are typical human serum BPA concentrations measurable and sufficient to be estrogenic in the general population? Food Chem Toxicol. 2013;62:949–63.
59. Organization WH (2011) Joint FAO/WHO expert meeting to review toxicological and health aspects of bisphenol A: final report, including report of stakeholder meeting on bisphenol A, Nov. 1–5, 2010, Ottawa, Canada.
60. Program NT. Carcinogenesis bioassay of Bisphenol A (CAS no. 80-05-7) in F344 rats and B6C3F1 mice (feed study). Natl Toxicol Program Tech Rep Ser. 1982;215:1–116.
61. Birnbaum LS. Environmental chemicals: evaluating low-dose effects. Research Triangle, NC: National Institute of Environmental Health Sciences; 2012.
62. Vandenberg LN. Low-dose effects of hormones and endocrine disruptors. In: Vitamins & hormones, vol. 94. Amsterdam: Elsevier; 2014. p. 129–65.

63. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee D-H, Shioda T, Soto AM, Vom Saal FS, Welshons WV. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev. 2012;33(3):378–455.
64. Mok-Lin E, Ehrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, Ye X, Hauser R. Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. Int J Androl. 2010;33(2):385–93. https://doi.org/10.1111/j.1365-2605.2009.01014.x.
65. Martínez M, Rovira J, Sharma RP, Nadal M, Schuhmacher M, Kumar V. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: a case study. Environ Res. 2017;158:566–75.
66. Yamada H, Furuta I, Kato EH, Kataoka S, Usuki Y, Kobashi G, Sata F, Kishi R, Fujimoto S. Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. Reprod Toxicol. 2002;16(6):735–9.
67. Souter I, Smith KW, Dimitriadis I, Ehrlich S, Williams PL, Calafat AM, Hauser R. The association of bisphenol-A urinary concentrations with antral follicle counts and other measures of ovarian reserve in women undergoing infertility treatments. Reprod Toxicol. 2013;42:224–31.
68. Saddick SY. Light and transmission Electron microscopic studies on subacute toxicity of Bisphenol A on the rat ovary. Anal Quant Cytopathol Histopathol. 2015;37(4):227–34.
69. Santamaría C, Durando M, de Toro MM, Luque EH, Rodriguez HA. Ovarian dysfunctions in adult female rat offspring born to mothers perinatally exposed to low doses of bisphenol A. J Steroid Biochem Mol Biol. 2016;158:220–30.
70. Kato H, Ota T, Furuhashi T, Ohta Y, Iguchi T. Changes in reproductive organs of female rats treated with bisphenol A during the neonatal period. Reprod Toxicol. 2003;17(3):283–8. https://doi.org/10.1016/S0890-6238(03)00002-9.
71. Chao H-H, Zhang X-F, Chen B, Pan B, Zhang L-J, Li L, Sun X-F, Shi Q-H, Shen W. Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway. Histochem Cell Biol. 2012;137(2):249–59.
72. Zhang T, Li L, Qin XS, Zhou Y, Zhang XF, Wang LQ, De Felici M, Chen H, Qin GQ, Shen W. Di-(2-ethylhexyl) phthalate and bisphenol A exposure impairs mouse primordial follicle assembly in vitro. Environ Mol Mutagen. 2014;55(4):343–53.
73. Lenie S, Cortvrindt R, Eichenlaub-Ritter U, Smitz J. Continuous exposure to bisphenol A during in vitro follicular development induces meiotic abnormalities. Mutat Res Genet Toxicol Environ Mutagen. 2008;651(1):71–81. https://doi.org/10.1016/j.mrgentox.2007.10.017.
74. Rivera OE, Varayoud J, Rodríguez HA, Muñoz-de-Toro M, Luque EH. Neonatal exposure to bisphenol A or diethylstilbestrol alters the ovarian follicular dynamics in the lamb. Reprod Toxicol. 2011;32(3):304–12.
75. Veiga-Lopez A, Beckett E, Salloum BA, Ye W, Padmanabhan V. Developmental programming: prenatal BPA treatment disrupts timing of LH surge and ovarian follicular wave dynamics in adult sheep. Toxicol Appl Pharmacol. 2014;279(2):119–28.
76. Gieske MC, Lawson C, Smith H, Murdoch B, Vande Voort C, Hunt PA. Fetal exposure to Bisphenol A causes meiotic defects and abnormal follicle formation in a primate model. Oxford, UK: Oxford University Press; 2011.
77. Migliaccio M, Chioccarelli T, Ambrosino C, Suglia A, Manfrevola F, Carnevali O, Fasano S, Pierantoni R, Cobellis G. Characterization of follicular atresia responsive to BPA in Zebrafish by morphometric analysis of follicular stage progression. Int J Endocrinol. 2018;2018:4298195.
78. Li Y, Zhang W, Liu J, Wang W, Li H, Zhu J, Weng S, Xiao S, Wu T. Prepubertal bisphenol A exposure interferes with ovarian follicle development and its relevant gene expression. Reprod Toxicol. 2014;44:33–40.
79. Altunbas K, Celik S, Yagci A, Akkaya OO. The effect of bisphenol A on Notch signaling pathway in the follicular development of neonatal rat ovary. In: World Congress of Reproductive Biology 2014. Bristol, UK: BioScientifica; 2014.

80. Moore-Ambriz TR, Acuña-Hernández DG, Ramos-Robles B, Sánchez-Gutiérrez M, Santacruz-Márquez R, Sierra-Santoyo A, Piña-Guzmán B, Shibayama M, Hernández-Ochoa I. Exposure to bisphenol A in young adult mice does not alter ovulation but does alter the fertilization ability of oocytes. Toxicol Appl Pharmacol. 2015;289(3):507–14.
81. Yön ND, Akbulut C. Histological changes in zebrafish (Danio rerio) ovaries following administration of bisphenol A. Pak J Zool. 2014;46(4):1153–9.
82. Hunt PA, Lawson C, Gieske M, Murdoch B, Smith H, Marre A, Hassold T, Vande Voort CA. Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey. Proc Natl Acad Sci. 2012;109(43):17525–30.
83. Brieno-Enriquez M, Robles P, Camats-Tarruella N, Garcia-Cruz R, Roig I, Cabero L, Martinez F, Caldés MG. Human meiotic progression and recombination are affected by Bisphenol A exposure during in vitro human oocyte development. Hum Reprod. 2011;26 (10):2807–18.
84. Susiarjo M, Hassold TJ, Freeman E, Hunt PA. Bisphenol A exposure in utero disrupts early oogenesis in the mouse. PLoS Genet. 2007;3(1):e5.
85. Lawson C, Gieske M, Murdoch B, Ye P, Li Y, Hassold T, Hunt PA. Gene expression in the fetal mouse ovary is altered by exposure to low doses of bisphenol A. Biol Reprod. 2011;84 (1):79–86.
86. Zhang H-Q, Zhang X-F, Zhang L-J, Chao H-H, Pan B, Feng Y-M, Li L, Sun X-F, Shen W. Fetal exposure to bisphenol A affects the primordial follicle formation by inhibiting the meiotic progression of oocytes. Mol Biol Rep. 2012;39(5):5651–7.
87. Aoki T, Takada T. Bisphenol a modulates germ cell differentiation and retinoic acid signaling in mouse ES cells. Reprod Toxicol. 2012;34(3):463–70. https://doi.org/10.1016/j.reprotox.2012.06.001.
88. Liu B, Zhou S, Yang C, Chen P, Chen P, Xi D, Zhu H, Gao Y. Bisphenol a deteriorates egg quality through HDAC7 suppression. Oncotarget. 2017;8(54):92359.
89. Xin L, Lin Y, Wang A, Zhu W, Liang Y, Su X, Hong C, Wan J, Wang Y, Tian H. Cytogenetic evaluation for the genotoxicity of bisphenol-a in Chinese hamster ovary cells. Environ Toxicol Pharmacol. 2015;40(2):524–9.
90. Allard P, Colaiácovo MP. Bisphenol a impairs the double-strand break repair machinery in the germline and causes chromosome abnormalities. Proc Natl Acad Sci. 2010;107 (47):20405–10.
91. Brieno-Enriquez M, Reig-Viader R, Cabero L, Toran N, Martinez F, Roig I, Garcia Caldes M. Gene expression is altered after bisphenol a exposure in human fetal oocytes in vitro. Mol Hum Reprod. 2011;18(4):171–83.
92. Ganesan S, Keating AF. Bisphenol A-induced ovotoxicity involves DNA damage induction to which the ovary mounts a protective response indicated by increased expression of proteins involved in DNA repair and xenobiotic biotransformation. Toxicol Sci. 2016;152(1):169–80.
93. Nah WH, Park MJ, Gye MC. Effects of early prepubertal exposure to bisphenol a on the onset of puberty, ovarian weights, and estrous cycle in female mice. Clin Exp Reprod Med. 2011;38 (2):75–81.
94. Shi M, Sekulovski N, Mac Lean JA, Whorton A, Hayashi K. Prenatal exposure to Bisphenol a analogues on female reproductive functions in mice. Toxicol Sci. 2019;168(2):561–71.
95. Bloom MS, Kim D, Vom Saal FS, Taylor JA, Cheng G, Lamb JD, Fujimoto VY. Bisphenol A exposure reduces the estradiol response to gonadotropin stimulation during in vitro fertilization. Fertil Steril. 2011;96(3):672–7.
96. Cao Y, Qu X, Ming Z, Yao Y, Zhang Y. The correlation between exposure to BPA and the decrease of the ovarian reserve. Int J Clin Exp Pathol. 2018;11(7):3375–82.
97. Berger RG, Hancock T, DeCatanzaro D. Influence of oral and subcutaneous bisphenol-a on intrauterine implantation of fertilized ova in inseminated female mice. Reprod Toxicol. 2007;23(2):138–44.
98. Grasselli F, Baratta L, Baioni L, Bussolati S, Ramoni R, Grolli S, Basini G. Bisphenol A disrupts granulosa cell function. Domest Anim Endocrinol. 2010;39(1):34–9.

99. Zhang Y, Gao J, Xu P, Yuan C, Qin F, Liu S, Zheng Y, Yang Y, Wang Z. Low-dose bisphenol a disrupts gonad development and steroidogenic genes expression in adult female rare minnow Gobiocypris rarus. Chemosphere. 2014;112:435–42.
100. Mlynarčíková A, Kolena J, Ficková M, Scsuková S. Alterations in steroid hormone production by porcine ovarian granulosa cells caused by bisphenol A and bisphenol A dimethacrylate. Mol Cell Endocrinol. 2005;244(1):57–62. https://doi.org/10.1016/j.mce.2005.02.009.
101. Zhou W, Liu J, Liao L, Han S, Liu J. Effect of bisphenol a on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. Mol Cell Endocrinol. 2008;283(1):12–8. https://doi.org/10.1016/j.mce.2007.10.010.
102. Mansur A, Adir M, Yerushalmi G, Hourvitz A, Gitman H, Yung Y, Orvieto R, Machtinger R. Does BPA alter steroid hormone synthesis in human granulosa cells in vitro? Hum Reprod. 2016;31(7):1562–9.
103. Peretz J, Flaws JA. Bisphenol a down-regulates rate-limiting Cyp11a1 to acutely inhibit steroidogenesis in cultured mouse antral follicles. Toxicol Appl Pharmacol. 2013;271 (2):249–56.
104. Banerjee O, Singh S, Prasad SK, Bhattacharjee A, Banerjee A, Banerjee A, Saha A, Maji BK, Mukherjee S. Inhibition of catalase activity with 3-amino-1, 2, 4-triazole intensifies bisphenol a (BPA)-induced toxicity in granulosa cells of female albino rats. Toxicol Ind Health. 2018;34 (11):787–97.
105. Santangeli S, Consales C, Pacchierotti F, Habibi H, Carnevali O. Transgenerational effects of BPA on female reproduction. Sci Total Environ. 2019;
106. Ziv-Gal A, Wang W, Zhou C, Flaws JA. The effects of in utero bisphenol a exposure on reproductive capacity in several generations of mice. Toxicol Appl Pharmacol. 2015;284 (3):354–62.
107. Wang W, Hafner KS, Flaws JA. In utero bisphenol a exposure disrupts germ cell nest breakdown and reduces fertility with age in the mouse. Toxicol Appl Pharmacol. 2014;276 (2):157–64.
108. Zhou C, Wang W, Peretz J, Flaws JA. Bisphenol a exposure inhibits germ cell nest breakdown by reducing apoptosis in cultured neonatal mouse ovaries. Reprod Toxicol. 2015;57:87–99.
109. Berger A, Ziv-Gal A, Cudiamat J, Wang W, Zhou C, Flaws JA. The effects of in utero bisphenol a exposure on the ovaries in multiple generations of mice. Reprod Toxicol. 2016;60:39–52.
110. Liu Y, Zhang Y, Tao S, Guan Y, Zhang T, Wang Z. Global DNA methylation in gonads of adult zebrafish Danio rerio under bisphenol a exposure. Ecotoxicol Environ Saf. 2016;130:124–32.
111. Liu Y, Yuan C, Chen S, Zheng Y, Zhang Y, Gao J, Wang Z. Global and cyp19a1a gene specific DNA methylation in gonads of adult rare minnow Gobiocypris rarus under bisphenol a exposure. Aquat Toxicol. 2014;156:10–6.
112. Zhang X-F, Zhang L-J, Feng Y-N, Chen B, Feng Y-M, Liang G-J, Li L, Shen W. Bisphenol a exposure modifies DNA methylation of imprint genes in mouse fetal germ cells. Mol Biol Rep. 2012;39(9):8621–8.
113. Santangeli S, Maradonna F, Gioacchini G, Cobellis G, Piccinetti CC, Dalla Valle L, Carnevali O. BPA-induced deregulation of epigenetic patterns: effects on female zebrafish reproduction. Sci Rep. 2016;6:21982.
114. Mahalingam S, Ther L, Gao L, Wang W, Ziv-Gal A, Flaws JA. The effects of in utero bisphenol a exposure on ovarian follicle numbers and steroidogenesis in the F1 and F2 generations of mice. Reprod Toxicol. 2017;74:150–7.
115. Moustafa GG, Ahmed AA. Impact of prenatal and postnatal exposure to bisphenol a on female rats in a two generational study: Genotoxic and immunohistochemical implications. Toxicol Rep. 2016;3:685–95.
116. Cotinot C, Mandon-Pepin B, Loup B, Amezaga MR, Lea RG, Sinclair KD, Rhind SM, Fowler PA In vitro exposre to environmental doses of BPA, MEHP and PCBs results in few transcriptions alterations in the fetal sheep ovary. In: Biology of Reproduction, 2013.

117. Ahsan N, Ullah H, Ullah W, Jahan S. Comparative effects of Bisphenol S and Bisphenol A on the development of female reproductive system in rats; a neonatal exposure study. Chemosphere. 2018;197:336–43.
118. Hill CE, Sapouckey SA, Suvorov A, Vandenberg LN. Developmental exposures to bisphenol S, a BPA replacement, alter estrogen-responsiveness of the female reproductive tract: a pilot study. Cogent Med. 2017;4(1):1317690.
119. Chen Y, Shu L, Qiu Z, Lee DY, Settle SJ, Hee SQ, Telesca D, Yang X, Allard P. Exposure to the BPA-substitute bisphenol S causes unique alterations of germline function. PLoS Genet. 2016;12(7):e1006223.
120. Nourian A, Soleimanzadeh A, Jalali AS, Najafi G. Effects of bisphenol-S low concentrations on oxidative stress status and in vitro fertilization potential in mature female mice. In: Veterinary research forum. Urmia, Iran: Urmia University; 2017. p. 341.
121. Zaid SSM, Othman S, Kassim NM. Potential protective effect of Tualang honey on BPA-induced ovarian toxicity in prepubertal rat. BMC Complement Altern Med. 2014;14 (1):509.
122. Mohamad Zaid SS, Kassim NM, Othman S. Tualang honey protects against bpa-induced morphological abnormalities and disruption of ERα, ERβ, and C3 mRNA and protein expressions in the uterus of rats. Evid Based Complement Alternat Med. 2015;2015:202874. https://doi.org/10.1155/2015/202874. Epub 2015 Dec 14. PMID: 26788107; PMCID: PMC4691614
123. Bilgi A, Abalı R, Bilgi PT, Şahin M, Tunçdemir M, Boran AB. The apoptotic effects of bisphenol a exposure on the rat ovary: an experimental study. Environ Sci Pollut Res. 2019;26 (10):1–6.
124. Mehranjani MS, Mansoori T. Stereological study on the effect of vitamin C in preventing the adverse effects of bisphenol a on rat ovary. Int J Reprod Biomed. 2016;14(6):403.
125. Lee C-T, Wang J-Y, Chou K-Y, Hsu M-I. 1, 25-Dihydroxyvitamin D3 modulates the effects of sublethal BPA on mitochondrial function via activating PI3K-Akt pathway and 17β-estradiol secretion in rat granulosa cells. J Steroid Biochem Mol Biol. 2019;185:200–11.
126. Zaid SSM, Kassim NM, Othman S. Tualang honey protects against BPA-Induced morphological abnormalities and disruption of ERα, ERβ, and C3 mRNA and protein expressions in the uterus of rats. Evid Based Complement Alternat Med. 2015;202874. https://doi.org/10.1155/2015/202874.

Chapter 4
Effects of Environment and Lifestyle Factors on Premature Ovarian Failure

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Abstract Premature ovarian insufficiency (POI) or primary ovarian failure is defined as a cessation of the menstrual cycle in women younger than 40 years old. It is strictly defined as more than 4 months of oligomenorrhea or amenorrhea in a woman <40 years old, associated with at least two follicle-stimulating hormone (FSH) levels >25 U/L in the menopausal range, detected more than 4 weeks apart. It is estimated that POI was affected 1 and 2% of women. Although 80% of POI cases are of unknown etiology, it is suggested that genetic disorder, autoimmune origin, toxins, and environmental factors, as well as personal lifestyles, may be risk factors of developing POI. In this section, we will discuss the influences of environmental and lifestyle factors on POI. Moreover updated basic research findings regarding how these environmental factors affect female ovarian function via epigenetic regulations will also be discussed.

Keywords Premature ovarian insufficiency (POI) · Environmental factor · Lifestyle · Epigenetic regulations

4.1 Introduction

Premature ovarian failure (POF) is used to define women who present with amenorrhoea, hypergonadotropic hypogonadism, and infertility in aged younger than 40 years. It is defined as amenorrhea of 4–6 months before the age of 40, meanwhile, the level of follicle-stimulating hormone (FSH) increased (FSH > 40 U/L), and the level of estradiol decreased [1]. However, given that POF can only represent the end stage of ovarian failure, it cannot well cover the long and variable clinical course of the disease. Therefore, in 2008, the American Society for Reproductive

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Medicine (ASRM) formally adopted the concept of "primary ovarian insufficiency," but since the primary is generally for secondary, here the "primitiveness" has caused a lot of confusion. Therefore, in 2016, the European Society of Human Reproduction and Embryology (ESHRE) published the latest POI Handling Guidelines, which changed the full name of POI to premature ovarian insufficiency (POI). In addition, the diagnostic threshold of FSH (40 U/L) was reduced to 25 U/L, which aims to detect women with POI earlier and achieve the purpose of early diagnosis and treatment. In the "Consensus of Experts on Hormone Supplementation Therapy for Early-onset Ovarian Insufficiency" published by the Menopause Group of the Chinese Association Obstetrics and Gynecology in December 2016, relevant domestic experts defined early-onset ovarian insufficiency as the ovaries of women before the age of 40. The clinical syndrome of active decline is characterized by menstrual disorders (such as menopause or rare menstruation) accompanied by high gonadotropins and low estrogen [2]. In the current research, the etiology of the chromosomal abnormalities or some autosomal gene defects, autoimmune ovarian damage, infectious factors, and iatrogenic factors has been identified. However, a large part of the etiology of POI is unknown [3, 4]. In recent years, with the changes in socioeconomic and living environment, the incidence of POI has been increasing, and the impact of environmental factors on reproductive health has been increasingly concerned. Some researchers believe that adverse environmental factors can decrease the female ovarian reserve [5, 6]. The environment exerts great influences on the occurrence and development of POI, which can be roughly divided into two aspects: natural environmental factors and social environmental factors.

In recent years, the influence of environmental factors on human health has also been concerned. Environmental, psychosocial, and lifestyle factors may accelerate the decline in ovarian reserve. With the development of industrialization and environmental pollution, laboratory data show that chemical environmental pollutants diffused in the environment have an adverse impact on mammals' ovaries [6]. Moreover, we need to concern about how these harmful factors interact with our body, what the underlining mechanisms are, and how we can better learn and understand their relationship, then finally to figure out an effective protocol to preserve ovarian function. Systems medicine may be a considerable method.

4.2 Environmental Factors and Premature Ovarian Failure

4.2.1 Natural Environmental Factors

With the development of society and the acceleration of industrialization, the impact of natural environment on fertility has become a part that cannot be ignored. When the substances created by humans greatly exceed the digestive power of nature in terms of quantity and potential toxicity, some chemicals can accumulate through the layers of the biological chain and eventually enter the human body, which, in turn, affects all aspects of the human body, these chemicals are called environmental

pollutants [5, 7–10]. Environmental endocrine disruptors (EEDs) are one of the higher levels of environmental pollutants, which can affect the normal functions of the human endocrine system and human health [11]. At present, cell models and animal toxicology experiments have confirmed that EEDs present deleterious effects on female reproductive systems. Consecutive exposure to low doses of EEDs can cause female reproductive system developmental disorders, reproductive system dysfunction, metabolic diseases, and even cancer [12–14]. Currently, environmental pollutants are considered to affect ovarian function in three ways: Endocrine, oxidative stress induction, and epigenetic modification [15]. Some environmental pollutants and their mechanisms are discussed below.

4.2.1.1 Endocrine Disrupting Chemicals (EDCs)

Environmental endocrine disruptors (EEDs) refer to a class of exogenous substances or their mixtures widely distribute in the environment that are known to affect the endocrine system of human and the function of natural hormones, leading to organ/tissue damage and diseases [16–18]. Depending on how long the ovaries are exposed to the environment containing EEDs, the effects of EDCs on ovarian function can be reversible or permanent. The toxic effect of EEDs on the ovary mainly means that EEDs can affect follicular development by directly acting on the ovary or the hypothalamus-pituitary-ovarian axis. At present, more than 800 EEDs have been found to interfere with the human reproductive system [11]. Several important environmental endocrine disruptors are discussed in detail below.

Phthalates (PAEs)

PAEs are a subgroup of fat-soluble compounds with a global output of more than six million tons per year [19]. They are currently used as plasticizers or softeners in industrial and personal care productions. Because of noncovalent conjugation with the products, phthalates are relatively easily released into the environment through direct release, migration, leaching, and abrasion, resulting in extensive and continuous exposure [20]. Thus, the presence of compounds in the environment has been paid considerable attention by scientists due to their potential effects on the ecosystem and human health.

The most representative substance in PAEs is DEHP, which is currently the most widely used plasticizer. DEHP is everywhere in life, and it can be exposed through oral intake, inhalation, or skin contact. Related animal experiments have found that continuous exposure to DEHP in mice can decrease the number of primary and secondary oocytes and increase the number of atretic follicles [21]. It has been reported that phthalates have adverse effects on female reproductive health, such as ovarian dysfunction. Animal studies showed that exposure to the most common phthalate, di (2-ethylhexyl) phthalate (DEHP), significantly decreases the quantity of primordial follicles and large antral follicles in ovaries of female mice [22, 23]. At

present, it is believed that PAEs affect ovary mainly through the influence of the follicle formation and development, primary follicle aggregation, ovulation, and ovarian development [24]. Continuous exposure of pregnant mice to mono-(2-ethylhexyl) phthalate (MEHP) can make oocytes damaged by changing the level of oxidative stress in oocytes, by accelerating follicle recruitment and depletion of the original follicle pool in offspring decreased numbers, leading to premature ovarian failure in offspring [25]. It was reported that the number and sex ratio of the offspring of mice was altered by DEHP exposed. Moreover, it showed that DEHP activated autophagy in the ovary by models of neonatal exposure and ovary culture, with increased expression of autophagy-related gene and autophagosomes. In contrast, 3-MA, an inhibition of autophagy, eliminated the adverse impact of DEHP on primordial folliculogenesis. In addition, AMPK was up-regulated by DEHP exposure in the ovary, while its inhibitor compound C reduced the expression of autophagy-related gene, which may partially recover the assembly of primordial follicle [26]. Continuous exposure of newborn mice to DEHP may accelerate ovarian follicle recruitment, thereby significantly reducing the number of primary follicles, primary oocytes, and secondary oocytes during puberty and adulthood [27]. Prepubertal mice exposing to DEHP can promote ovarian somatic cell apoptosis and oxidative stress, leading to a significant decrease in the proportion of sinus follicles [22]. Clinical studies have found that MEHP can also affect the development of human ovaries. Studies by Muczynski et al. have shown that women exposed to MEHP at 10–4 mol/L for 72 h during 12–72 weeks of pregnancy can lead to disorders of lipid and phospholipid synthesis in the body. At the same time, MEHP can affect the morphological development of the ovary by affecting the nuclear receptor signaling pathway [28]. At present, we think that the possible mechanism is that MEHP induces the generation of ROS, which in turn generates a series of oxidative stress and interferes with glutathione peroxidase (GPX) and superoxide dismutase 1 (SOD1) expression, finally affecting follicular function [29]. The above research shows that it is clear that PAEs have damaging effects on the ovaries of all ages. How to prevent POI caused by PAEs has become a public concern. It is the best way to find PAEs sources in the environment as early as possible and to stay away from PAEs sources as soon as possible.

Polychlorinated Biphenyls (PCBs)

PCBs are a fat-soluble environmental hormone with good insulation properties and are mainly used in the production of electronic transformers, capacitors, and coolants. PCBs are a type of persistent organic pollutants. Because of their bioaccumulation and long-term residual nature, PCBs were included in the Human Carcinogens Catalogue by the International Agency for Research on Cancer (IARC) in 2013. PCBs were gradually banned all over the world, but due to their fat-soluble and hard-to-degrade properties, they have been widely used in the environment and food chain. PCBs can stably exist in the environment, and their metabolites are not easily eliminated in the human body. They are mainly accumulated in the adipose

tissue of the human body, and the half-life is about 10–15 years [30, 31]. Studies have proven that exposure to PCBs can have reproductive toxicity in the development of female reproductive systems and ovaries. Because PCBs have a phenol structure, they can compete with estrogen, androgen, and progesterone receptors in the human body, which affects the function of the human reproductive system [32]. Murat et al. found through cell experiments that hamster ovary cell line (CHO-K1) exposed to different concentrations can affect PCBs' proliferation, metabolic capacity, and adhesion, resulting in different degrees of damage to ovarian cell membranes [33]. Animal experiments have found that PCBs exposure during pregnancy can reduce ovaries weight in born mice, and that most of the anal follicles are blocked, delaying vaginal opening, and causing external urogenital tract abnormalities. That is, PCBs can directly affect the ovaries and adversely affect female puberty by changing the morphological and functional development of female reproductive systems [34]. Clinically, maternal exposure to PCBs can produce reproductive toxicity to offspring through the placenta, and the direct effects on the offspring's ovaries are more severe than the mother's, which can be manifested in the reduction of offspring ovarian weight, follicular atresia, and even POI [35]. The above evidence proves that PCBs have reproductive toxicity, and the damage to women and their offspring ovaries is severe. Since PCBs are not easy to eliminate in the human body, they have accumulation characteristics. Avoiding contact with PCBs is currently an effective way to prevent PCBs from ovarian toxicity.

Bisphenol A (BPA)

BPA is widely used in the polycarbonate plastics and epoxy resins industries. It is detectable in several consumer products, such as water bottles, food containers, dental sealants, and thermal receipts [36–39]. Due to the high content of bisphenol A in daily products, bisphenol B exposure is widespread in humans. The main methods are diet, inhalation, and skin absorption [36, 40, 41]. With the acceleration of the pace of life and work, fast food and takeaways have become the norm for people, and this change has become the most common way for people to contact BPA. Because of the capacity to interfere with the synthesis, metabolism, and activity of several endogenous hormones, BPA belongs to endocrine disruptor. It can affect the endocrine system by interacting with multiple nuclear receptors, including estrogen, androgen, glucocorticoid, and thyroid hormone receptors [42–44]. Health concerns have been aroused regarding the hormone-like BPA. Substantial evidence indicated that BPA exposure increased susceptibility to human reproductive functions [45, 46]. Animal studies have found that pregnant mice exposure to BPA from week 1 can cause a significant decrease in the number of starting and growing follicles in the offspring, the number of atretic follicles increased significantly, and the process of meiotic division of the egg cells was also inhibited [47]. At the same time, the experimental results have also been confirmed in vitro experiments of human embryonic egg cells [48]. The experiment exposed newborn rats to BPA and

found a large number of ovarian cysts appeared in adult ovaries, with a significant decrease in fertility and progesterone levels, and increase in serum testosterone and E2 levels, suggesting that BPA may affect the hypothalamic-pituitary-ovarian axis (HPOA) function which interferes with the production of sex hormones. High-dose exposure of BPA during the period of newborn is potentially related to the development of polycystic ovary syndrome and premature ovarian failure in adulthood [49, 50]. Some researchers have found through epidemiological studies that human BPA exposure can reduce the number of sinus follicles and oocytes, and even POI, leading to infertility [51]. At present, it is believed that the reproductive toxicity mechanism of BPA is that it can regulate the expression of genes through epigenetic regulation, thereby causing reproductive toxicity to the human body. BPA exposure in early human life can affect the appearance of endocrine signaling pathways and some important signaling pathways. The genetic process, the exposure of BPA during pregnancy, can change the steroid hormone synthesis genes and microRNAs related to gonadal differentiation and follicle synthesis in the offspring, and eventually destroy the offspring's fertility [52].

4.2.1.2 Smoking/Polycyclic Aromatic Hydrocarbons (PAHs)

As we all know, smoking is harmful to our health. Substantial studies have confirmed that various harmful substances of tobacco can cause different degrees of ovarian damage. The most studied component is polycyclic aromatic hydrocarbons (PAHs). PAHs are mainly created by the burning of minerals or plastics, but because of the large number of smokers, cigarettes have become the main source of people's exposure to these compounds. Smoking is now considered to be detrimental to reproductive and pregnancy outcomes and may increase the risk of idiopathic POI. In earlier years, the adverse impact of smoking has been summarized by several comprehensive reviews [53–55]. It is also supported by most of the studies which were excluded from the meta-analysis, compared with nonsmokers, the prevalence of infertility is higher, the fecundity is lower, and the time to conception is increased in smokers. Related animal studies have shown that when pregnant rats are exposed to smoking, the ovarian granulosa cells of their offspring significantly apoptotic, and AFC decreased, indicating that smoking during pregnancy will cause a decline in offspring ovarian reserve and even POI [56]. Some studies have suggested that the mechanism of POI caused by smoking is mainly apoptosis and oxidative stress, and the consumption of follicle reserve cannot be stopped immediately after quitting smoking [57]. The above points have been confirmed in the clinic that smoking causes women to reduce anal follicle count [57]. Lowering AMH (anti-Muller hormone) levels also makes early menopause [58]. Related epidemiological surveys show that smokers have a higher prevalence of infertility, lower fertility, and a longer conception time than nonsmokers [59]. Many of the above mechanisms can explain that compounds in tobacco smoke can act on the ovary in many different ways. It is clear that the exposure to tobacco smoke can lead to a reduction in the quantity of primary and mature follicles in the ovary, an increase of apoptosis and

oxidative stress in sinus follicles and oocytes, and significant estrogen and progesterone level changes affect normal ovarian function and even cause POI.

4.2.1.3 Organophosphorus Pesticides

Chemical pesticides are the main means of controlling crop diseases and insect pests and ensuring high and stable yields of crops. Among them, organophosphorus (OP) pesticides have stability and lipophilicity and have a slow degradation rate. They can be kept in the environment for several years. In this way, they exist widely in the food chain. Organophosphorus pesticides have been used widely as agricultural and household pest control agents for almost five decades and persist in our water resources, fruits, vegetables, and processed food as health and hazardous environmental compounds. With the widespread use of organophosphorus pesticides in China, people's awareness of the harms of organophosphorus pesticides, the low-dose, long-term, chronic exposure of organophosphorus pesticides to human health, especially the health effects of pregnant women's exposure on offspring, has caused China's extensive attention. There are mainly two approaches of environmental exposure to OPs and PYRs, food consumptions with pesticide residues and application of pesticides indoors [60–62]. Animal studies have shown that OPs exposure has adverse effects on female reproductive functions, such as decreased steroid hormones and fertility, disordered estrous cycles, and restricted follicle cells, which might finally lead to POI [63–65]. Other animal studies found that OPs exposures could disturb estrous cycles, leading to a reduction in the number of estrous cycles and the duration of proestrus, estrus, and metestrous with a concomitant prolonged diestrous phase, and ultimately caused infertility [65–67]. Another potential mechanism might be involved in follicular development. Animal experiments show that using organophosphorus pesticides commonly used in agriculture: monocrotophos, dimethoate, methyl parathion, and 90 rats were poisoned and then experimented. The results show that all three organophosphorus pesticides can significantly reduce the concentration of binding proteins, total lipids, phosphates, and cholesterol in the ovarian cytoplasm and cell membrane, and the ovaries show a deteriorating change. In other words, these three organophosphorus pesticides can cause ovarian changes and induce reproductive toxicity. In addition, studies have reported that organophosphorus pesticides (dimethoate) can significantly reduce ovarian weight in mice with compensatory ovarian overgrowth [68]. In the clinic, some researchers investigated 3103 women living in farms who used pesticides. Compared with women exposed to other pesticides, women exposed to organophosphorus pesticides experienced prolonged menstrual cycles and irregular menstrual cycles, indicating that organophosphorus pesticide exposure can cause women's menstrual cycle disorders and may reduce fertility [69]. Prolonged contact with or consumption of foods containing organophosphorus pesticides will cause ovarian hypofunction and even POI. Some researchers believe that the possible mechanism of the toxic effect of organophosphorus pesticides on ovarian tissues is: directly toxic to the ovaries or by interfering with hormone levels at any stage of

the HPOA, inducing degeneration of ovarian organs. But the developmental and reproductive toxicity of organophosphorus pesticides is a multi-path and very complex process, and the mechanism is not clear yet. We should avoid exposure to organophosphorus pesticides to prevent decline in reproductive function, and also conduct in-depth research on its mechanism.

4.2.1.4 Radiation

Radiation is widely present in the environment in which we live, but prolonged exposure to radioactive and electromagnetic radiation may cause harm to humans or other animal bodies. With the development of technology and communication, electromagnetic radiation widely exists in the environment in which we live and continues to affect the functions of our tissues and organs. It has become the fourth largest pollution after air pollution, water pollution, and noise pollution. Radioactive radiation is mainly divided into natural origin and iatrogenic origin, here mainly refers to iatrogenic radiation used for treatment. At present, the effects of radioactive radiation and electromagnetic radiation on reproduction have become the focus of our current research. Common electromagnetic radiation sources in life are: radar and radio transmission systems, televisions and smart phones, most household appliances, subway trains, equipment of radiofrequency induction and dielectric heating, microwave medical and various electrical processing, large electrical power generation stations, and transmission equipment, such as high and ultra-high voltage power lines. The main radioactive and electromagnetic radiation will be discussed in detail below.

X-Ray Radiation

Iatrogenic radiation mainly comes from radioactive diagnosis and treatment. It mainly refers to clinical X-ray examination, CT scan, various angiographic examinations, and nuclear medicine examinations. Radioactive radiation has been proven to endanger male reproductive health, affect male reproductive endocrine, reduce male fertility, and lead to increased male infertility [70, 71]. In recent years, the prevalent of cancer was gradually increased, as well as radiotherapy is one of the main treatments. Due to work, illness, or accidents receiving large doses or long-term radiation, the impact of radiation on women's ovarian function has gradually attracted scholars' attention. The radiation damage to the ovaries has been confirmed by a large number of investigations, and radiation can accelerate the onset of menopause and cause permanent infertility [72]. Researchers found that exposure to high doses of radiation can lead to infertility, while low doses of radiation can lead to a partial depletion of the initial follicle reserve, leading to premature ovarian failure [73]. It is reported in the literature that the cumulative dose of 14.3 Gy received in 30 years can also cause premature ovarian failure [72]. Other researchers have demonstrated through animal experiments that as the dose of X-ray radiation

increases, the number of follicles in different stages in the rat's body decreases. 0.4~1.0 Gy), which is down-regulated, that is, radiation can induce apoptosis of ovarian granulosa cells, which may be dose-dependent [74]. The damaging effects of radiation therapy on ovarian are associated with the dose, field, and age. The dose that causes half of the follicle loss in humans is 4 Gy, and the sensitivity of the ovaries to ionizing radiation gradually increases with age. In women under 40, ionizing radiation requires 20 Gy for premature ovarian failure, while older women only need 6 Gy [75]. Except for nuclear contamination and radiotherapy, the general population has few chances to receive a large amount of low-dose radiation, but the health hazards of radioactive radiation at regular inspection doses cannot be ignored. In clinical practice, we should try to reduce the number of radiological examinations as much as possible, and if necessary, we should protect them to ensure that the cells of the reproductive organs are not damaged or to minimize the damage.

Electromagnetic Radiation/Mobile Phone Radiation

Modern devices such as mobile phones, laptops, microwave ovens, and induction cookers have brought great convenience to people's lives, and brought electromagnetic radiation to the environment in which we live. Many studies have confirmed that electromagnetic radiation has a negative influence on multiple organs of the body. With the widespread and long-term use of mobile phones in the national life of our country, the adverse effects of electromagnetic radiation on the reproductive functions have received widespread attention. A clinical epidemiological investigation confirmed that cell phone radiation may be a risk factor for the decrease of human ovarian reserve function. Animal experiments show that cell phone radiation can reduce the drosophila ovary and reduce reproductive capacity, which may be related to early and intermediate DNA breaks in oocytes [76]. There are also experiments that show that electromagnetic radiation can reduce the quantity of ovarian follicles in the offspring of pregnant mice, can affect the maturation of rat's oocytes so that reduce the fertility of oocytes and even cause infertility, and can break single- and double-stranded DNA in cultured rat granule cells [77, 78]. Some researchers have imitated the transmission frequency and average power density of mobile phones, observed a common mobile phone frequency of 900 MHz, simulated a large average power density of 370 $\mu W/cm^2$ before switching on, and irradiated the female rats' ovarian capillaries for 4 h per day-mutual aid the effect of ataxia-telangiectasia mutated (ATM) protein expression and damage ovarian function. The experimental results show that the ATM protein of rat ovarian granulosa cells is significantly enhanced after cell phone irradiation, suggesting that cell phone radiation may cause damage to the DNA of rat ovarian granulosa cells and corpus luteum cells, which enhances the expression of ATM protein, the "monitor" of DNA damage and activates the repair system to repair the damaged cells further, so that the ovarian granulocytes and luteal cell ATM proteins of rats are significantly enhanced after irradiation. It is speculated that the cause of impaired ovarian function caused by cell phone radiation may be related to DNA damage and ATM increased protein

expression [79]. ATM is a gene that has a priming effect in the repair of cellular DNA damage and can respond to priming effects on DNA damage caused by many other biological, physical, chemical, and other factors. It plays a very important regulatory role in radiation-damaged DNA repair, can initiate DNA repair early in the damage, regulate the cell cycle, induce apoptosis, and maintain DNA and genome stability. At present, it is believed that ubiquitous cell phone radiation will cause ovarian function damage in female rats. After cell phone irradiation, the level of serum E2 in rats during estrus will decrease, the diameter of follicles and corpus luteum will decrease, and the number and layer of granulosa cells will decrease, indicating impaired ovarian function. The mechanism may be related to affecting the expression of ovarian DNA and its repair ATM protein. Long-term exposure to mobile phone electromagnetic radiation can cause ovarian function damage and even POI in female rats. At present, there is no unified radiation dose standard for the occurrence of electromagnetic radiation and POI, and research is limited. At present, prevention is the main focus, and further research and exploration are needed.

4.2.1.5 Noise (Repeated Exposure to Noise May Be Perceived as a Succession of Stressors, and Therefore, Noisy Environments Could Lead to a State of Chronic Stress)

The development of modern science and technology has provided great convenience to human life, and at the same time, it has also caused noise problems. Noise is a hazard of invisible pollution, and it has become the third-largest city pollution after air pollution and water pollution. Long-term exposure to noise can cause damage to the hearing system, and can also affect the regulation of the HPOAs, causing female genital endocrine systems to malfunction, which in turn affects the pregnancy process, pregnancy outcomes, and offspring development—hazardous factors. The impact of noise on female reproductive function has attracted scholars' attention. Among them, some researchers believe that the abnormal menstrual cycle of female workers is closely related to occupational noise exposure. It has been reported that long-term exposure to high-intensity noise can strongly stimulate the central nervous system and make it dysfunction, endocrine disorders, then cause women's menstruation and ovarian dysfunction. At present, it is believed that noise can make the human cerebral cortex and central nervous system often in a state of tension. Over time, it can easily cause human endocrine disorders and an imbalance of estrogen in the body. Similar results have been obtained in animal experiments. Noise can cause reproductive abilities in mice, which may be due to the increased secretion of corticosteroids in rodents and the failure of implantation of fertilized eggs [80].

4.2.1.6 Climate Warming/Heat Stress

With climate change and global warming, people are paying attention to the reproductive effects caused by heat stress. In tropical or subtropical countries, high

ambient temperature seems to be a risk factor contributing to decreased fertility in cattle, although in regions with temperate climates, the involvement of heat stress is also well documented in this phenomenon [81, 82]. Heat stress influences reproductive functions, such as ovarian functions and embryonic development [83]. Mammalian ovarian follicles composed of oocyte and the surrounding granulosa cells (GCs) and theca cells. GCs and theca cells produce signals and hormones which ensure oocyte competency to develop into the blastocyst stage [84, 85]. Normal proliferation and differentiation of GCs are crucial for optimal follicular growth, oocyte development, ovulation, and luteinization [86]. It has been reported that heat stress had adverse effects on GCs, such as oxidative and endoplasmic reticulum stress, and cells apoptosis [87–89]. Oxidative stress is a consequence of reactive oxygen species (ROS) imbalances within cells. Excessive ROS overload cellular antioxidant defenses and cause damage of lipids, proteins, and DNA, leading to normal cell function disrupting and cell death via apoptosis or necrosis [90]. In addition, studies have suggested that heat stress significantly reduced the GCs proliferation, with decreased proliferation marker gene PCNA expression. In summary, heat stress induces oxidative stress, cell apoptosis, and inhibited proliferation, which triggers the NRF2 mediated oxidative stress and endoplasmic reticulum stress response by GCs. At present, it is clear that the damage of ovarian function caused by heat stress can cause ovarian reserve decline and even POI. In today's increasingly global warming, prevention of ovarian function from climate change is another problem we face.

4.2.1.7 Conclusion and Suggestions

As mentioned above, with the continuous development of technology and agriculture, environmental pollutants exist in the natural environment in which we live. As with the substances described above, environmental pollution is a critical threat of the reproductive health of animals and humans, and its harmful effects can affect endocrine as well as reproductive functions. Among them, PAEs, PCBs, BPA, and other environmental endocrine disruptors exist anywhere in the environment of our daily life. They mainly interfere with some ovarian hormones in the body and abnormally activate specific receptors, causing ovarian endocrine system disorders, leading to follicle apoptosis or insufficiency, changes in the function of the HPOA or granule cell, and even directly activate some apoptosis-related genes, leading to follicular apoptosis. Many substances in cigarettes can seriously affect human health. In terms of ovarian function, smoking can cause abnormal apoptosis of ovarian cells, affect ovarian estrogen expression, affecting the menstrual cycle and follicular development, finally reduce the ovarian reserve. Organophosphorus pesticides may cause abnormal ovarian function by affecting the function of the HPOA. Even accidental exposure to organophosphorus pesticides in normal agricultural labor will adversely affect female reproductive health. In addition, some physical factors such as radiation, noise, and global climate warming may also cause adverse effects on female reproductive health by mediating apoptosis, causing DNA damage,

and affecting endocrine functions. The above research findings suggest that women should increase their awareness of protection. Many harmful factors are usually ignored in daily life, which may have adverse consequences on reproductive health and increase the risk of reproductive-related diseases. For women who are usually exposed to the above said harmful substances, it is recommended to strengthen their personal protection, reduce the chance or dose of exposure, make sure take effective personal protection at work, and take effective measures as soon as possible after exposure to those harmful factors, in order to prevent their further harming in the body.

4.2.2 Social Environmental Factors

It is currently believed that environmental, social-psychological, and lifestyle factors may accelerate the decline of ovarian reserve function. In recent years, with the changes in socioeconomic, living environment and lifestyle, POI has been increasing. The influence of socio-psychological factors on female reproductive health has also received increasing attention. With the acceleration of the pace of life in modern society, increased work pressure, shortened sleep time, women in the reproductive period are in a state of tension and anxiety for a long time, which affects the level of HPOA secretion, affects ovarian function, leads to ovarian reserve decline and even POF. A systematic review found that inadequate workplace control, poor social support, hostility, depression, and anxiety are associated with coronary heart disease, and there is ample evidence that psychosocial factors affect healthy neuroendocrine pathways. Relevant research shows that psychosocial factors are related to ill health, and following social gradients can explain some or all of the social ill-health phenomena, and it is also a biologically reasonable explanation. Some researchers believe that the direct impact of physical conditions (noise, radiation, air and water pollution) on health in the natural environment is as important as the health impact of relative deprivation on the psychosocial impact. Related animal experimental models have found that chronic mental stress can cause ovarian reserve in female rats to decline; that is, psychological factors are also important factors that cause POI. At the same time, as an advanced animal, people are affected by socio-psychological factors such as economic strength, social status, and interpersonal relationships in a complex social network. There is evidence that these factors affect health and their prevalence is affected by the socioeconomic structure and the impact of people's place in it.

4.2.2.1 Education Level

Since the reform and opening up, our country's economy, science, and technology have developed rapidly, so the per capita education level has been greatly improved, and the improvement of women's education level is particularly obvious. Changes in

fertility levels are inseparable from social development, and the impact of education on fertility behaviors and fertility concepts has been recognized. The socioeconomic statue is composed of several factors, which includes education, family income, and social prestige. A trend has been found that increasing menopausal age was associated with increasing educational levels. Compared with low, primary/vocational education level, women with university degrees had a later median age of menopause [91]. And the specific mechanism is currently still not clear [92]. Although education associated with income, it is a factor that is beyond the economic dimension of socioeconomic position. It is also a reflection of investment in social capital, expressing lifestyle, social paths accumulated, and the investment in social capital made by a society. In addition, several studies have found that women with longer educational years have menopause later than women with fewer years of education [91, 93, 94]. With the vigorous development of education, women have more and more opportunities for higher education, and the number of women with higher education has increased. The higher the education level of women, the age of their first marriage will be delayed as the quantity of years of education increases, and the age of childbearing will also be delayed. The extension of the length of education will inevitably lead to the delay of childbearing age. Strong scientific data support that growth, fertility gradually decreased, and the female fertility rate began to decline significantly from the fourth decade of life. That is to say, because of the extension of the length of education, the delay of women's reproductive age has caused a decline in fertility to a certain extent, which is inconsistent with the effects of the educational level mentioned above on the age of menopause and menopause. Current research suggests that an increase in education can delay the arrival of menopause, but when it comes to the impact of education on POI, it is believed that the extension of the length of education delays the reproductive age, leading to a decline in fertility, combined with women at work. In the long-term career, there is a lot of psychological pressure on life and work, which may lead to women's POI.

4.2.2.2 Socioeconomic Status

Since the reform and opening up, the problem of imbalance in China's economic development has become more serious. In a society, health is related to socioeconomic level, that is, income. In addition to meeting basic needs, the socioeconomic level is also linked to social status and psychology. Generally speaking, the socioeconomic level is equal to the amount of wealth. Compared with those with lower socioeconomic levels, people with financial ability can provide themselves and their families with good material conditions. Of income are associated with lower subordinate rights, higher autonomy and control, and less job insecurity [95]. Multiple researches indicated that lower socioeconomic levels are involving in earlier menopause [96–99]. However, the potential mechanisms underlying the effects of socioeconomic factors accumulated on human ovarian physiology during women's lives are still unclear. But at present, it is believed that the adverse social and economic conditions of children have a greater impact on the age of natural

menopause than on the adulthood of adults. The possible reason is that children's socioeconomic deprivation leads to imbalances in children's nutrition and growth and development. The main theory concerns the low socioeconomic deprivation suffered by women in childhood, especially diet deprivation. The result is reduced oocyte development, which will have an adverse effect on the duration of estrogen and progesterone [100]. Early childhood growth in adverse socioeconomic conditions will affect linear growth, as well as menopausal age. The fact of fertility distinguished by social/occupational class is well known. High social status may be associated with lower fertility [101]. There is a study showing a great significant link between fertility and the social status of the married couples, with mean family size increasing by about one child between social classes I and II together and V based on husband's class and by 0.65 based on wife's social class [102].

However, there are relatively few studies on the impact of social class on fertility. Whether social class and socioeconomic status lead to POI needs further study.

4.2.2.3 Chronic Stress

Current research suggests that after undergoing various adverse stimulations, the body of human creates a series of reactions through regulating the neuroendocrine system, which is called "stress" by scientists. With society development, women are more and more important for society. Meanwhile, women also bear more and more responsibilities and stress. Their bodies are often live with stress. Clinical studies have shown that the common biological response of women suffering from extra stress is that when human body faces the source of stress by the sense system, it will trigger various defensive responses, including neuroendocrine system, biological behavior, and so on. It is currently believed that chronic stress is a crucial environmental factor for ovarian and sexual dysfunction, infertility, and other common diseases [103]. Studies have reported that the incidence of POI is closely associated with depression, anxiety, and other negative emotions. According to foreign epidemiological surveys, about 43% of American POI patients have reported a history of depression, and 26% of them have been diagnosed with depression 5 years before diagnosis [104, 105]. The study showed that chronic psychological stress, including anxiety and depression, can impair women's ovarian function, leading to female reproductive endocrine disorders. In recent years, with the application of chronic unpredictable mild stress (CUMS) in depression models, many research studies have begun to explore the feasibility of chronic unpredictable mild stress methods in the establishment of POI models. In order to analyze the effects of chronic psychological stress on ovarian reserve, Fu Xiaoyan and others successfully established a POI model by CUMS in animal experiments [106]. They raised the rats separately and repeated the following set of CUMS procedures: wet pad 24 h, behavior limit 2 h, fast for 24 h, water for 24 h, forced ice water to swim at 60 °C, 4 °C for 5 min, reverse day and night for 24 h, noise interference for 12 h, tail suspension for 30 min, foot shock for (30 V) seconds. Stimulation was given randomly every day, $\leq$35 days. The results showed that CUMS-treated rats lost weight, prolonged estrus

cycle, changed ovarian morphology and reduced follicle count. The study found that on the 35th day after the POI model was established, the rat's ovaries showed significant atrophy. The main histological changes were as follows: severe fibrosis of the ovarian matrix, thickening of the cortex, and structural disorders; the quantity of follicles decreased while the quantity of atretic follicles increased significantly; the corpus luteum showed fibrosis and increased in number. At the same time, CUMS treatment reduced the levels of E2, AMH, and GnRH in the serum of rats, while the opposite level of FSH increased. Barra et al. demonstrated that chronic cold stress may cause ovarian dysfunction or POI in adult female rats, which is characterized by prolonged or lack of physiological regulation and reduced secretion of estradiol and progesterone [107]. In addition to reducing the quantity of primary and secondary follicles of the ovary, the follicle-stimulating hormone (FSH) receptors expression had been reduced, and the sexual development in young rats had been delayed. Chronic cold stress may also cause ovarian dysfunction in adult female rats [108]. As the above studies have shown, chronic stress may be an important factor in the progress of POF.

4.3 Lifestyle Factors on Premature Ovarian Failure

Compared with other immobile etiologies such as genetic and environmental factors, learning and understanding the influence of modifiable lifestyle factors in POI seem more meaningful in the daily life of reproductive women. The most established and well-learning lifestyle factor associated with POI is smoking, in addition, body mass, alcohol and caffeine intake, diet, sleep quality, oral contraceptive, and physical activity were suggested as the potential factors. Therefore, understanding when and how those lifestyle factors affect the ovarian functions of reproductive women and cause POI can provide more healthy suggestions for women to adjust their lifestyle and help medical staff work out better healthy proposals.

4.3.1 Smoking

Smoking is an independent risk factor of chronic obstructive pulmonary disease, various cancers, cardiovascular disease, and many other diseases. Smoking has become a global health problem, which threatened millions of lives in the world. According to a recent cross-sectional study, the smoking rate in postmenopausal women was 11.8%. In China, though the rate of smoking women was relatively lower, the exposure of the passive smoking rate was quite considerable [109]. Apart from those diseases mentioned above, smoking can injure the reproductive system of women, especially the ovarian function, and causes gynecological diseases or even infertility [110].

As a public health problem, smoking is the best understanding and most established lifestyle risk factor of POI. Considerable effort has been devoted to describing the whole picture on the influence of smoking in POI. In this part, we will discuss the influence in the aspect of smoking status, intensity, accumulative dose, start and quit age of smoking, and passive smoking with the updated basic research findings.

4.3.1.1 Smoking Status

In those studies which estimated the association between smoking and menopausal age, smoking status was the primary or basic point to discuss. Though not all the studies were detected POI directly, the conclusion that current smoking is a risk factor of an earlier age of menopausal was always consistent.

A meta-analysis comprising 15 studies found that current smokers were owning an earlier weighted mean difference (WMD) by nearly 1 year compared with never smokers, though with a substantial heterogeneity [110]. However, in the subsequent subgroup analysis by taking region into consideration, the heterogeneity in this meta-analysis was reduced and could be accepted. In addition, this study analyzed the adjusted hazard ratio and suggested that current smoking was with a 33% increased risk of undergoing postmenopausal at a given age. The result of this study suggested that current smoking does have a relationship with an earlier age of natural menopausal. However, there was no significant difference found in former smoking, partially explained by a few studies reported at this point.

In another more recent article, a cross-sectional study involving 207,231 post-menopausal women from seven countries has shown that compared with who never smoke, current smokers faced a higher risk of premature menopause (defined as menopausal age <40 years) with the relative risk ratios (RRRs) were 2.05 [111]. At the same time, the RRRs of other age groups before reference group were all beyond 1, suggesting that current smoking was one of the risk factors of earlier menopausal age. Additionally, it is also found that the RRRs of premature menopause in former smokers were 1.13 ($P = 0.006$), which were lower compared with current smokers but still with a significant difference, indicating that former smoking may still have influence on age of menopause. However, this conclusion was inconsistent with some former studies, which thought that former smoking had no effect or just slight on women menopausal age [112, 113]. But one of the potential mechanisms linking smoking to earlier menopause supported the first opinion [111]. This hypothesis explained cigarette smoke might induce expression of Bcl2, one of the genes of apoptosis, and its related protein in oocytes, which could lead to oocyte apoptosis and then cause early menopause. This influence was permanent and irreversible, so that might explain the increasing RRRs of the earlier age of menopause in a former smoker. Though controversy in the effect of former smoking exists, it is clear that smoking cessation benefits the age of natural menopause.

4.3.1.2 Smoking Intensity

Researches usually used the number of cigarette smoking per day to estimate the smoking intensity [111]. At this point, the question most interesting to the researchers is whether there is a dose–response effect between smoking intensity and its effect on menopausal age, and the answer seems to be yes [114].

Animal researches have shown a dose–response relationship between nicotine and the impairment in follicle growth [4]. Some studies categorized smoking intensity into only two groups with a certain number, such as 10 or 20 cigarettes per day [113, 115, 116]. In those studies, researchers easily found that the odds ratio or risk ratio of earlier menopausal age was increased in the higher-intensity group. However, some more detailed studies that categorized smoking intensity into more groups found that the dose–response relationship still existed but there is no significant difference or linear trend [114]. In the recent cross-sectional study [111], the intensity of smoking was categorized into three groups: 1–9, 10–19, and more than 20 cigarettes per day, and found that the adjusted RRRs of POI were 1.59, 2.23, and 2.71 respectively, which indicated a clear dose–response effect between smoking and POI. This relationship also observed in early menopause and existed similar results in former smoking [115].

More cigarette smoking each day means more toxic substances will be absorbed by out body. The present evidence strongly suggested higher smoking intensity increased the risk of earlier menopause and even has shown a direct relationship in POI. Such results suggest that controlling the dose of cigarettes each day also benefits the smoking women in prevention of early menopause and POI.

4.3.1.3 Smoking Duration and Accumulative Dose

Compared with smoking intensity, smoking duration may better reflect the long-term effect of cigarette smoking. Similar to smoking intensity, a dose–response relationship also observed in duration. In a cross-sectional study in 1995 [117], researchers found that the hazards ratios of early menopause among women aged 45–54 in the smoking duration of $<$10 years, 10–20 years, 20–30 years, and more than 30 years were 1.05, 1.13, 1.34, and 1.84, respectively. This result has shown a gradually increasing risk of earlier menopausal age with the prolonged duration of smoking. Such findings were also observed in a prospective cohort study published in 2016 [115]. Moreover, this study also estimated the effect of smoking in infertility and found a similar result with early menopause. However, this study just compared the age of menopause before and after 50 years, so that cannot particularly reflect the effect on early menopause or POI. In the cross-sectional study in 2018 [111], researchers found the adjusted RRRs of POI among current smokers who smoking at least 10 years before menopause were 9.22, meaning more than 9 times of POI risk in women smoking at least 10 years. Among 11–14 and 15–20 years of smoking duration, the adjusted RRRs sharply increased to 14.34 and 15.58, indicating worse

situations happened in longer smoking duration. Since the RRRs of POI in smoking duration were distinctly higher than other estimated points, this article suggested that the smoking duration could be a reliable predictor of early menopause or POI. However, this effect was lower in the former smoker but still showed a dose–response relationship.

The accumulative dose of smoking is another point that can reflect the long-term influence of smoking cigarettes. There were usually two approaches to measure the accumulative dose, which were pack-years [111] (assessed with the number of cigarette packs multiply the duration years) and the total number of cigarette smoking (assessed by the quantity of cigarettes per day multiply the total smoking days) [118], both of them were combined the quantity of cigarettes and the duration of smoking into consideration. In accordance with expectations, the results of the assessment in accumulative dose were similar to smoking duration in both cross-sectional studies published in 1995 [117] and 2018 [111]. In the study in 1995, the accumulative dose was categorized into four categories of pack-years <10, 10–19, 20–29, and more than 30, and the hazards ratios were 1.10, 1.28, 1.33, and 1.87, respectively. The risk of early menopause was increased by 87% when the pack-years reach 30. In the study in 2018, authors categorized the pack-year into <5, 6–10, and 11–15, which found that the adjusted RRRs of POI were increased gradually both in current smokers and former smokers. Although the RRRs were lower than smoking duration, accumulative dose has better representativeness and reflected the long-term influence in smoking.

Given that the dose–response relationship both existed in duration and the accumulative dose of smoking, which means smoking has a long-term and accumulative effects at the age of menopause and increases the risk of POI, to quit smoking as soon as possible and ever control the numbers and times of smoking cigarette could reduce the risk of earlier menopausal and POI.

4.3.1.4 Start and Quit Age of Smoking

Although the age to start and quit smoking may associate with smoking duration, which means an earlier start or a later quit age of smoking may link to a longer smoking duration, there is value to estimate the effect of it in early menopause and POI when considering that there are more and more people start smoking in teenage age and, at the same time, assessing the benefits of smoking cessation directly.

Evidence proved that earlier age to start smoking increases the risk of earlier menopause, especially at an age under 15 years old. Although a study from Korea published in 2015 suggested there was no difference in menopausal age between age to start smoking under and over 20 years old [116], more well-designed studies showed a different result. A study which involved 2123 participators used the group of start smoking under 20 years old as a reference and found that the women in groups of start smoking among 21–24 years old and over 24 years old held a relatively lower adjusted odds rate of early menopause, which were 0.68 and 0.59, respectively [117]. This finding suggested a nearly half of risk reduction in later start

smoking age. In another study published in 2016 [115], the odds rate of menopausal age under 50 years old in women start smoking under 15 years old was higher than other groups but was not shown a linear reduced tendency as the increase of start smoking age, which means the risk of earlier menopause was not different when the start smoking age was over 15 years old. The cross-sectional study published in 2018 involving 207,231 participators found the direct evidence between POI and start age of smoking [111], which showed a reduced adjusted relative risk ratio of POI at a later start age of smoking in both current and former smokers. Those researches suggested that the adverse effect of cigarette smoking may affect teenager worse, since it is an important period of reproductive system growing. Thus, school and family education is much important for teenagers to get far away from cigarette smoking.

In terms of the quit age of smoking, few articles have mentioned about. However, the existing evidence suggested longer interval years between smoking cessation and menopause reduced the risk of earlier menopause. In a prospective study following up 3545 women for 21 years published in 2012 [113], researchers estimated the risk of early menopause in former smoking women categorized into four groups as never smoking, quit smoking before and after 14 years follow-up, and smoking in the whole 21 years follow-up, and results found that compared with never smoking, the population of women menopause occurred before 45 years old in other three groups was significantly higher. At the same time, the adjusted hazard ratios were higher in women who quit after 14 years and never quit during follow-up (1.41 and 1.61) compared with those who never smoke and quit before 14 years follow-up (1.00 and 0.91). In another study, researchers found that the odds ratios of early menopause in women who stop smoking more than 10 years before menopause were decreased down to 0.14. In addition, the recent cross-sectional study has shown the RRRs of POI in the former smokers who quit smoking in years of 1–5, 6–10, and 11–15 before menopause were 1.71, 1.42, and 0.84, respectively [111]. Those evidence suggested that for former smokers, as time went by, the adverse effect of smoking cigarette on the menopausal age could reduce. Though longer quit years of smoking mean longer smoking duration and reduced accumulative dose effect, the quit years could be an important variable when discussing the influence in former smoking. When quitting smoking over 10 years, the risk of early menopause and POI nearly reduced to a normal level; this may explain why adverse effects cannot be found in some researches and highlight the benefit of earlier quit smoking.

4.3.1.5 Passive Smoking

Passive smoking, also known as second-hand smoking, threatens more females compared to active smoking. For women, it is more likely to expose to second-hand smoking rather than active smoking, especially women in China. According to a recent report of the prevalence of smoking in Chinese Sichuan province, the proportion of female smoker was 2.4%, which was lower than in many western countries. However, the prevalent rate of smoking men was 50.3%, and the rates of

people exposed to passive smoking were up to more than 50% in homes and workplaces, where women and children were always the victims of passive smoking [119]. In this aspect, the effect of passive smoking in women's reproductive functions is worthy to figure out.

It is still controversial whether passive smoking increases the risk of earlier menopause, and lack of researches directly links its effect to POI. A small prospective study published in 1999 observed no decrease in natural menopausal age of the passive smoking women [120]. A later study held the same opinion, though they found a 1.56 odds ratios of early menopause in women who grew up with smoking mother or father, it is not statistically significant [121]. In contrast, another observational study found a slight adverse effect of second-hand smoking in earlier menopause [122]. In a cross-sectional study involving 7596 US women at the age of 25–50, researchers used logistic regression model to assess the odds of earlier age at menopause, and the results found that the odds ratios were increased in both smoking and passive smoking women [123]. The odds ratios of earlier menopause in Hispanic passive smoking women were 19.08, higher than black and white women. This research also found that the difference of odds between active smoking and passive smoking was slight, which is according to a small case–control study in 1986 [120], though it did not justify the conclusion that effects of active and passive exposure are equivalent. Those findings suggested that it is necessary to distinguish second-hand smokers from former smokers when analyzing the effects of cigarette smoking. Further researches need to clarify the effect of passive smoking in earlier menopause and POI.

According to an investigation [119], passive smoking usually takes place in homes, workplaces, and some specific public places through inhalation. Thus, smoking control in those places is quite important. Women in reproductive age also need to avoid passive smoking in those places consciously since evidence had found adverse effects in earlier menopause.

Except inhalation, another way to exposure second-hand smoking is transplacental. Animal studies had shown that exposure to cigarette in utero is toxic to the ovarian development. A recent cohort study followed up 2852 women from 1991/1992 until 2010 and found that in utero cigarette exposure was not a risk factor of earlier menopause [124]. However, the hazard ratios of earlier menopause were higher in smoking women who had in utero exposure to cigarette compared with smoking women who had not. This finding suggested that women would better quit smoking during pregnancy, for the benefit of the child.

In summary, smoking has an association with the age of menopause and increases the risk of POI. There is a dose–response relationship between the risk of earlier menopausal age and smoking intensity, duration, and the accumulative dose of smoking. Earlier start age of smoking increases the risk of earlier menopause and POI while longer quit years before menopause could reduce the risk. It is still controversial in the effect of passive smoking on menopausal age, and further investigations need to clarify their relationship.

4.3.2 Alcohol and Caffeine Consumption

Alcohol and coffee are both common daily drinking. Heavy alcohol consumption is associated with many diseases, such as acute or chronic gastritis, liver diseases, and sleep disorder [125], while caffeine consumption can cause intensity, anxiety, hypertension, and osteoporosis. In those studies seeking the relationship between lifestyle factors and menopause, alcohol and caffeine consumption has been usually estimated as confounding factors, which means the potential influences of alcohol and caffeine intake may exist on the women's age of menopause. However, there are no studies that reveal their effects on POI. In this part, we will discuss the relationship between alcohol and caffeine consumption and women's menopausal age and assess their value of ovarian prevention.

4.3.2.1 Alcohol

Due to the levels of dehydrogenase enzymes, an essential enzyme that breaks down alcohol in our body is relatively lower in female compared to male, coupled with the higher fat/water ratio of the female body, alcohol levels in woman body rise quickly than man after ingestion, which means females are more vulnerable to alcohol's harmful effects [126].

Whether alcohol consumption is related to the onset of menopause or not is still controversial. Moreover, in those studies proved the existing relationship, how does the effect exert is also controversial.

In a cross-sectional study published in 2007 involving 2123 postmenopausal women found that early menopause was not significantly associated with alcohol or coffee consumption [120]. An early study published in 1996 found that moderate consumption of alcohol was correlated with serum E2 levels ($r = 0.61$) and associated with delayed menopausal age [125]. In a longitudinal study published in 2006, researchers also found that women who drank alcohol 5–7 days/week experienced 2.2 years of delayed onset of menopause, compared with women who did not drink [127]. For women who drank at least 1 day/week, the delayed onset of menopause was shifted to 1.3 years. Moreover, according to a recent systematic review and meta-analysis which included 22 articles, low and moderate alcohol intake (less than one drinking per week and three or fewer drinks per week) was associated with later menopausal age, RR = 0.60 and 0.75, respectively [128]. Also when analyzed with dose, similar results were found in women who were drinking 0–8 g/day and 16 g/day. That evidence indicated that alcohol intake may contribute to later menopausal age.

However, in a recent cross-sectional study from Korea involving 940 women, researchers found that the onset of menopausal age was earlier in mild to moderate drinkers (<30 g/day) and heavy drinkers (>30 g/day) compared to women who were never drinking in all three adjusted model [129]. When assessed with scores of Alcohol Use Disorders Identification Test (AUDIT), similar results were observed.

Those results indicated that alcohol consumption might lead to an earlier age of menopause. This conclusion was in accordance with a study when adjusted the result by smoking and caffeine only, but inconsistent with most previous studies. The authors analyzed that the difference may be explained by the difference in ethnicity, types of alcohol consumed, climate, and cultural determinants.

Most previous studies trend to aggress with alcohol consumption delay the age of menopause. In this aspect, the association between POI and alcohol seems limited. However, evidence exists that alcohol was associated with earlier menopausal age, and animal study had proved that alcohol was associated with oxidative stress, which may indicate ovarian damage. In this aspect, alcohol consumption may be a potential risk lifestyle factor of POI. Those controversial reports indicate that the effects of alcohol consumption have not been clearly revealed and need further investigation to clarify.

4.3.2.2 Caffeine

There were rare researches investigated the relationship between drinking coffee and the age of menopause, and most of the existing studies concluded that there is a negative association between these two events. A cross-sectional study published in 2007 found that the odds ratio of early menopause in women who were drinking coffee more than four cups per day was 1.64. However, this result was not significant [121]. Two studies have been evaluated the relation between caffeine and ovarian age [130, 131], neither of them found a positive or independent association of caffeine consumption and those indicators of ovarian such as anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH). According to a cross-sectional survey, caffeine consumption was positively associated with vasomotor symptoms bother in postmenopausal women [132].

In summary, neither alcohol nor caffeine and their effects on menopausal age were well understanding. The opinion of the effects of alcohol is still controversial, and there was no evidence indicated that the relationship existed between caffeine consumption and early menopause or POI.

4.3.3 Daily Diet and Nutrition

As the development of human society, the dietary habit of people has changed. The hard period about hungry and shortage of food support has become the past. Food support is rich and choices are various in developed countries, and, on the other hand, obesity and eating disorders are increasing. However, in some developing countries and rural area, malnutrition is still a major problem. Both lack and over food consumption can lead to healthy problems [133]. In terms of female reproductive health, the Dutch famine during 1944–1945 had proved that shortage of caloric intake in early childhood postponed the natural menopause [134]. Though lack of

studies revealing the association of daily diet and POI, researchers have found that many kinds of food and nutrition are associated with menopause age. In this part, we will discuss some well-learned dietary factors and their influence on age of menopause and seek for a proper approach to protest ovarian function based on daily diet.

4.3.3.1 Carbohydrate

A study published in 2006 concluded that low-fat, high-carbohydrate diet didn't influence the age of menopause [135]. However, some researchers also found that high carbohydrate intake was associated with later menopause age. According to a recent research published on Journal of Sichuan University, low carbohydrate and dietary fiber intake were related to higher odds of POI [136]. This case–control involved 70 women in POI group and 224 women in the other. Using multivariate analysis, researchers found that the odds ratio of POI in low level of dietary carbohydrate intake (<267.74 g/day) was 11.65, significantly higher than the high level of carbohydrate intake (>332.66 g/day). Those evidences indicated that low carbohydrate intake may be associated with POI; however, the opinion on the effect of high carbohydrate intake was still inconsistent.

4.3.3.2 Fruit and Vegetable

A large prospective research involving 33,054 Shanghai women has shown a relation of the onset of menopause [94]. They found that high level fruit intake (>383.2 g/day) was associated with delayed menopause. This effect was contributed to the antioxidant content in fruit. Another study learning from Australian women also supported the finding [137]. They analyzed the data of a large prospective cohort of which initial aim was to investigate the association between diet and occurrence of cancer, and found that fruit intake was related to menopausal age ($r^2 = 0.09$, $P = 0.004$), and a survival analysis proved women with more fruit intake (>5 times/day) had a longer reproductive age compared to women who took less (<3 times/day). This finding was also supported by a recent study in China and they found that intake of fresh fruit more than 1 day per week reduced the risk of early menopause and POI [138]. These existed evidences suggested that frequent intake of fruit benefited reproductive span prolonging.

However, the opinion of effects of vegetable on menopausal age was inconsistent. The Shanghai study found that menopausal age was not associated with vegetable intake [94], whereas more studies suggested that higher vegetable intake also benefited in prolonging the onset of menopausal age due to the antioxidant effect.

4.3.3.3 Protein

A longitudinal research of nearly 5000 German women found that higher intake of protein was associated with later menopausal age, which was aggressed with the prospective study in Shanghai women [94]. A recent study found that protein from vegetable sources reduced the risk of early menopause, whereas no similar effect was observed with animal protein intake [36]. The study published in Journal of Sichuan University found that low level of protein intake had a high OR (1.894) of POI though it did not find significant difference due to a relative small size of study [136]. According to a recent study in China done on women, intake of sea food (1–3 days each week) and fresh eggs (>4 days each week), which were high protein content, was inversely associated with early menopause [138]. These results suggested high protein intake may decrease the risk of earlier onset of menopause; however, the association with POI needs further research.

4.3.3.4 Fat

Controversies exist in the effects of fat intake on age of menopause. Studies had found positive, negative, and no relationship of fat intake and menopause intake [134]. The type of fat may cause the difference. Studies found that high intake of polyunsaturated fats was associated with earlier menopausal age, while total fat and saturated fat intake has no effect on menopause. Compared to other two major nutrient substances, carbohydrate and protein, little was known about the association of fat and menopausal age.

4.3.3.5 Other Daily Food

According to the previous published literature, other common daily food is also related to the age of natural menopause. A case–control study involving 160 participators found that POI patients had a lower frequency of both having red meat and fish compared to the control group [139]. Other study found that eating meat 1–3 times per week slightly increased the risk of later menopause [138]. However, another study did not find any difference on the onset of menopause age among women with intake of different level of red meat. One study found that low dietary fiber intake was associated with increasing odds of POI [136]. Dietary fiber intake had been thought to interrupt the enterohepatic circulation of sex hormones, causing the lower circulating estrogen concentrations in vegetarian women. Cereal products and soy products were connected with an earlier age of natural menopause [139]. In addition, higher caloric and cholesterol intake reduced the risk of earlier menopause [134].

4.3.3.6 Micro-Nutrient

A large cross-sectional study from China found that women with vitamins intake reduced nearly half of odds of POI compared with women who were not [138]. However, this article did not point out the details about what kind of vitamins had been investigated. Association had been proved between vitamin D and anti-Müllerian hormone (AMH), used as a parameter of ovarian reserve, and VD may trigger the production of AMH [39]. Other study has shown that vitamin C was correlated with the age of menopause ($r^2 = 0.06$). However, a previous study calculated the dose of dietary intake and found that none of middle or high intake of vitamin A, C, D, or E was associated with early menopause, but found an increased odds in high calcium intake [139].

A longitudinal study from Australia found that β-cryptoxanthin intake was correlated with the age of menopause ($r^2 = 0.105$) [138]. β-cryptoxanthin is an antioxidant, and is known to exist in the ovarian tissue. Dietary β-cryptoxanthin intake is primarily from fruit and vegetables. In this study, survival analysis proved that women with high β-cryptoxanthin intake (≥568 mcg/day) had a later menopausal age compared with low intake women(≤132 mcg/day). Using multiple linear regression analysis, the article found a model indicating that the 100 mcg increment of intake per day was associated with a delay for 6 weeks in the age of menopause. Those findings could explain the effects of green and yellow fruit and vegetables on delayed menopause age.

In summary, the balance of enough food support and nutrient is essential to women ovarian function. Enough carbohydrate, protein, and caloric support are important to a normal onset of menopause. Fruit, vegetable, and other foods containing antioxidant substance are helpful in protection of ovarian tissue and reduce the risk of earlier menopause. However, the effect of fat was still controversial and needs further investigation. High protein content food such as meat, sea food, and egg seems to be associated with later menopausal age, as well as enough vitamin support. Fiber, soy, and cereal products seem to have an adverse influence.

4.3.4 Body Mass

Body mass had been thought of associated with menopause for a long time; however, conclusions among studies remained controversial. Both overweight and underweight had been reported to be associated with earlier menopause, and overweight was also associated with later menopause. Whereas some studies reported that there was no relationship between body mass and menopausal age. These controversial reports may be explained by: (1) different race/ethnicity of subjects among studies; (2) different study designs and number of participators; (3) whether considering the confounders or not and different ways to estimate those confounders;

(4) difference of statistical analysis [140]. In this part, we will compare the different opinions among relevant studies.

4.3.4.1 Earlier Menopause Associated with Underweight

A recent study from Korea found that the menopausal age among women with body mass index (BMI) <25 was earlier than with BMI ≥25 [131]. A study from China also found that the odds of early menopause increased 55% (OR = 1.55) in underweight women [138]. The odds of POI was slightly increasing but not statistically different. A large prospective study involving 78,759 American women published in 2017 found that women who were underweight (BMI 18.5–22.4 kg/m^2) in the early or mid-adulthood had increased significantly 30% risk for early menopause [141]. This study also found a non-linear, J-shaped relation between BMI and the risk of early natural menopause, which found a reduced OR in women with overweight, indicating that overweight may be related to later menopause. This finding was agreed with a meta-analysis published in 2010 [140], which found that increased BMI prudently associated with later menopausal age, and explained that the relationship might be associated with higher estrone production in the adipose tissue in obese women.

4.3.4.2 No Relationship Between Body Mass and Menopausal Age

A meta-analysis including seven published data concluded that no clear association was proved between being overweight or obesity and menopausal age [110]. A small prospective study which only included 185 women also found no difference of menopausal age between women with BMI more than and <27.3 kg/m^2 [142]. This finding was agreed with two large cross-sectional surveys from Canada [143] and Norway [144] which categorized BMI into four categories of underweight, normal, overweight, and obese. The study from Norway observed a relative high OR (1.95) of early menopause in underweight women (BMI < 18.5 kg/m^2) but not with statistical significance.

4.3.4.3 Earlier Menopause Associated with Overweight

There were less studies supporting this opinion. A multi-ethnic population study found that the mean BMI was significantly higher in women with POI (29.3 ± 68.4 kg/m^2) compared with non-POI women (26.7 ± 66.3 kg/m^2; $P = 0.001$), which indicated that POI patients may trend to overweight [115]. And this study also proved a slight increasing OR of POI with multivariate models after adjustment for site, age, and socioeconomic status among Caucasian and African American women with higher BMI. Data from two European cohort studies showed that obesity is a determinant of earlier timing of menopause, with 1.3 HR in women

with BMI >30 kg/m^2. In this study, another result worth to mention is that HR was higher in women with BMI <18.5 kg/m^2 (HR = 1.7, CI 0.942–3.060), however, without statistical significance [144].

In summary, though such controversial opinions of body mass and menopausal age exist and need more further research to explain, keeping a normal BMI is important since there were no studies suggesting earlier or later menopause was associated with women with normal BMI.

4.3.5 Physical Activity

Similar to body mass, the comments on physical activity and age of menopause were controversial. Previous studies tend to suggest that heavy physical activity is associated with later menopausal age since the evidence that heavy physical activity can suppress gonadotropin-releasing hormone and gonadotropin activity, subsequently descend serum estrogen levels and lead to anovulatory or irregular menstrual cycles is supported by the fact that athletes usually have a later age of menarche and increased incidents of anovulation and amenorrhea, and have a reduced luteal phase with the mean and peak progesterone levels [143]. It's proved that fewer cumulative menstrual cycles are related to a larger reserve of oocytes and therefore later menopause. Data from a Japanese prospective cohort study [145] and a UK cross-sectional study [146] both published in 2012 supported this comment. At the same time, few or no physical activity was thought to be associated with earlier menopause. According to a meta-analysis, onset of menopause happened nearly one-third of a year earlier in physically inactive women compared with moderately or highly active women [138].

However, a recent prospective cohort study from the USA published in 2018 found physical activity was not associated with early menopause [147]. This large prospective study included 107,275 women followed up from 1989 to 2011 and observed 2786 women experienced early menopause. MET hours/week (hours of physical activity multiply by its metabolic equivalent score) was used to assess the intensity of physical activity. Cox proportional hazard models were used to calculate hazard ratios and adjusted by multiple confounders. Relationship of physical activity and menopausal age was not found in any models. The results also did not find that early menopause was associated with proper and arduous activity in adolescence and young adulthood. A nation-wide longitudinal study in Canada [148] and a cross-sectional study in Norway [121] published previously both supported the comment, though they didn't specifically lean on physical activity.

In addition, some study also found that high level of physical activity increased the risk of early menopause. A cross-sectional study from the USA involving 50,678 found that the risk of earlier onset of menopause slightly increased with women who have regular strenuous exercise (HR = 0.96) [146]. Another more recent cross-sectional study from China found that the risk of early menopause was increased

among women with middle and high level of physical activity (OR = 1.20 and 1.30, respectively) compared to women with low level [138].

Those inconsistent comments may be due to the different methods of physical activity intensity assessing. In some studies, MET hour/week was used, while hour/week was used by other study. And some studies just simply categorized it into low, middle, and high level or regular and no regular. In addition, association with different types of exercises was not better investigated. Some studies concluded that relationship just existed with extreme high level of activity; however, physical activity of participators from most study did not reach such high level. Different study designs may also part of the reason. And there is possibility that relationship of physical activity and menopausal age is none or limited, and the controversy was caused by other confounded factors.

4.3.6 Other Potential Factors

4.3.6.1 Sleep Quality

Though sleep quality is associated with many health problems and more and more concerns had been put into its effects on menopausal age, few studies had investigated their relationship. A recent study estimated the relation between rotating night shift work and menopausal age and found some correlation [149]. This large prospective cohort study followed up 80,840 women from Nurses' Health Study since 1991 until 2013. They found that recent 2 years and accumulative more than 10 years of rotating night shift work were risk factors for earlier onset of menopause. Authors thought the adverse effects may be resulted from circadian disruption and stress brought by rotating night shift work. A previous study found that sleep quality was associated with follicle-stimulating hormone (FSH) secretion [150]. Faster rate of FSH change was related to longer sleep duration with poor sleep quality. These studies indicated sleep quality may be a potential involving factor of onset of menopause but this effect needs further investigation.

4.3.6.2 Oral Contraceptive

Little was known about the relationship of oral contraceptive and onset of menopause. A cross-sectional study published in 2001 found oral contraceptive use reduced the risk of earlier menopause [115]. However, a multi-ethnic longitudinal study did not find any relationship of oral contraceptive and POI among Caucasian, African American, and Hispanic women [149].

4.3.6.3 Marital Status

Few studies also found that onset of menopause may be related to marital status. The multi-ethnic longitudinal study from the USA found single women had a higher risk of POI compared to married women [149]. The OR of POI among single Caucasian women was 1.8; however, it was not increased among single African American and Hispanic women. A cross-sectional study from Norway published in 2007 found that the risk of early menopause was higher among widows (OR = 1.89), but found no significant increasing risk among unmarried and divorced or separated women [121]. However, another study found that the risk of earlier menopause was increased among separated, widowed, and divorced women (HR = 1.27) [115]. Marital status may be related to other lifestyle factors and reproductive factors which may be associated with onset of menopause. Married women seem to have a stable status and a lower risk of earlier menopause [138, 151].

4.3.6.4 Reproductive Factors

Some reproductive factors may also influence onset of menopause. Studies found that earlier age of menarche may be related to earlier onset of menopause. A recent large cross-sectional study found that the odds of POI and early menopause increased among women who experienced menarche before 12 years (OR = 2.14 and 1.59, respectively) [138]. This study also found that more live births reduced the odds of POI and early menopause. The OR of POI was 0.38 in women who had three or more live births compared to women who just had one, which means multiple live births may prolong the reproductive age [138]. Those findings were agreed with another cross-sectional study from Norway [121]. In addition, age of first live birth had been thought to be associated with onset of menopause; however, few studies had mentioned about or the effect was slight.

4.4 Systems Medicine and Premature Ovarian Failure

As we discussed previously, various environmental and lifestyle factors influenced the onset of menopausal age and even increased the risk of POI. However, more important things are how these harmful factors interact with our body, what the underlining mechanism is, and how we can better learn and understand their relationship, then, finally to figure out a healthy protocol to protect ovarian function. Systems medicine may be a considerable method.

Systems medicine, initially appearing in the 1990s, refers to a new modern medical systems using systems-based theories and approaches to study and understand the complexity of human diseases, and integrating the substantial fundamental and clinical data from traditional and modern medical researches. Systems medicine

puts human into a whole ecosystem, investigates the interaction among human diseases, personal behavior, and environment via medical computer modeling and multiples molecular medical bio-technology, which combines theories of endocrine and immune systems regulation, as well as metabolism organ system [152].

Ovarian reserve refers to count of primordial follicles (PMFs) in ovary. Reduction of ovarian reserve, which occurs when folliculogenesis was interfered, is one of the risk factors of earlier menopause and POI, especially regarding those environmental and lifestyle factors [15, 138, 153]. Therefore, a normal folliculogenesis is essential for women reproductive health.

Folliculogenesis begins with primordial germ cells in the early embryonic development. Primordial germ cells separate from extraembryonic mesoderm and finally migrate into genital ridges, where primordial germ cells develop and become PMF. Although there is various evidence, on the contrary, the current agreement is female germ cells are not developed later in life. Therefore, the PMFs form a fixed pool called the ovarian reserve. Most of the PMFs are dormant under the negative intra-oocyte regulators. During each menstrual cycle, a group of PMFs are recruited and activated, however, only one dominant follicle (DF) finally experiences ovulation, and the rest of follicles (subordinate follicle, SF) undergo apoptosis during the period of primary follicle, secondary follicle, or antral follicle [154].

Multiple endocrine hormones, cytokines, and signal pathways are involved and make up a complex communication network, which regulates the folliculogenesis [155]. Any mistakes of the communication network may lead to follicular apoptosis or over activation, which will decrease the ovarian reserve that may cause POI [15]. For example, deletion of gene Pten, a PI3K-negative regulator, in mouse ovary results in activation of the whole PMF pool and therefore premature ovarian failure.

Many environmental and lifestyle factors affect onset of menopausal age mainly via interrupting the folliculogenesis [15, 156]. Although some of the mechanisms were not interpreted completely, several approaches of interruption may be involved, namely (1) activating apoptotic gene and opening dead signal pathway in ovary lead to increasing follicular apoptotic, (2) producing more reactive oxygen leads to oxidative stress, (3) affecting the functions of endocrine system, especially the effectiveness of estrogen, (4) causing DNA damage or epigenetics change which may lead to transgenerational effect, (5) affecting the communication between oocyte and other cells, and so on. In this part, we will summarize and categorize the possible mechanism of main environmental and lifestyle factors which we have discussed above.

4.4.1 Environmental and Lifestyle Factors Activated the Apoptotic Gene

The number of PMFs refers to ovarian reserve which reflects the function of ovary. PMFs in the ovary may experience one of the three possible fates: (1) remain

quiescent, (2) be activated and join the growing pool of follicles and then either go through atresia or finish their developmental process up till ovulation, and (3) die directly from their dormant state [153]. Normally, the PMFs pool is enough for a woman to experience a healthy reproductive life. However, when the PMFs endure extra apoptosis, the ovarian reserve may reduce, which may lead to early menopausal age or POI [15].

One of the most established apoptosis signal pathways is caused by polycyclic aromatic hydrocarbons (PAHs), one of the components of cigarette. PAHs come from incomplete combustion. In addition, smoking, contrived source of PAHs, which contain more than 100 chemical substances, is the process of manufacture or handicraft, fossil fuel and trash burning, and traffic pollution [157].

PAHs is a well-known cancerogenic substance [158]. In ovary, PAHs activate the aromatic hydrocarbon receptor (AhR), which presents on the surface of granulosa cells and is one of the transcription factors, and then induces expression of the apoptosis-promoting gene Bcl2-associated X protein (BAX), subsequently leading to apoptosis of granulosa cells and follicular atresia. This intracellular cell-dead pathway was first identified by Tiina Matikainen and his colleagues in 2001 [159]. Based on the previous study reporting that ovary is capable of metabolizing PAH compounds [160], they found that intraperitoneal injection of a prototypical PAH in female mice significantly increased ovarian BAX mRNA levels, and BAX protein accumulated in primordial and primary follicles. They further demonstrated an increased level of BAX protein in ovary was both necessary and sufficient to target female germ cell death and BAX gene deficient oocytes were resistant to PAH-induced apoptosis. Finally, they found that human primordial and primary follicles exposure to PAH increased the occurrence of degenerating oocytes, coupled with the increasing BAX accumulation. This pathway of follicular apoptosis supports the comment that smoking causes a permanent and irreversible influence to ovarian function [111]. BCL2, an anti-apoptosis protein, was reportedly decreased in ovaries of mice exposed to mainstream smoke in another study [161]. In addition, PAHs induce or up-regulate the expression of cytochrome P450 enzymes, which may convert PAHs into even more toxic molecules and decrease the serum estrogen levels [5].

In addition to PAHs, di (2-ethylhexyl) phthalate (DEHP), one kind of phthalates also shows an effect on reducing ovarian reserve [15]. Phthalates are used in the manufacture of plastics for various industrial applications. It has been reported that exposure to different dose of DEHP could reduce the number of primordial follicles in ovary of mice. Sen et al. exposed mice to dibutyl phthalates at 0.1 mg/kg/day for 10 days and resulted in decreased antral follicle numbers, coupled with increased mRNA encoding pro-apoptotic genes (BAX, BAD, BID) [162]. Another study conducting oral exposure of adult mice to DEHP (20 or 500 mg/kg/day) every day for 10 days resulted in decreased number of primordial follicles and increased BAX/BCL2 ratio in primordial follicles, indicating an adverse effect of phthalates on ovarian reserve [23]. Moreover, some kinds of pesticide were reported a similar effect. Park et al. administered 5–500 μg/kg dose of simazine, one of the pesticides,

to pregnant mice, and resulted in apoptosis of granulosa cells in the first generation with downregulation of anti-apoptotic and proliferation genes [163].

Soy and soy-based products, such as tofu, soy milk, and soy infant formula content soy isoflavones (SIFs), have many helpful health effects, such as the prevention of cancers and cardiovascular diseases and the prevention of osteoporosis in menopausal and postmenopausal women. However, Wang et al. orally administered SIFs to female rats 50, 100, or 200 mg/kg body weight from weaning until sexual maturity, resulting in increased percentage of apoptosis follicles, coupled with increased mRNA expression of apoptosis-related genes, including Bax and Fas [164]. This finding indicates that intake of soy and its productions may lead to reducing ovarian reserve through Bax or Fas mediated apoptotic signaling pathways.

In addition, exposure to X-ray had been reported to be associated with reproductive health. The Bcl-2 protein was observed increasing in ovary of rat as the growing of X-ray intensity. Bcl-2 protein can inhibit cytochrome C released from mitochondria into cytoplasm, thus suppress apoptosis.

In summary, PAH induces apoptosis of follicles via activating BAX gene.DEHP, simazine, SIFs, and X-ray exposure result in follicular apoptosis and observing relative gene and proteins change, however, their actual relationship requires further investigation to clarify.

4.4.2 Environmental and Lifestyle Factors Induce Oxidative Stress

Oxidative stress refers to a disturbance in the prooxidant–antioxidant balance in favor of the former, which may lead to various health problems [165]. Oxidative stress occurs under several pathological situations, which produces extra reactive oxygen species (ROS) or reactive nitrogen species (RNS) in human body. In contrast, there are two kinds of antioxidant systems resisting the oxidative stress, enzymatic antioxidant system (including superoxide dismutase, glutathione peroxidase, and catalase) and non-enzymatic antioxidant system (including ergothioneine, vitamin C and E, glutathione, melatonin, alpha-lipoic acid, and so on). In normal situation, the balance between oxidant and antioxidant systems is essential for human body. When oxidant substances overproduce, antioxidant system fails to eliminate those extra ROS and keep this balance, then oxidative stress happens [166].

Oxidative stress is proved to be associated with diabetes and human senescence. In ovary, three types of damages may response to follicles loss: (1) those extra ROS or RNS can induct lipid peroxidation reaction, damage the biological membrane, and finally lead to structural and functional change in cells; (2) ROS also causes protein damages, including signaling pathway and structural proteins, which may lead to gene expression changes or cellular structural changes; (3) ROS causes DNA

damages that lead to gene expression changes [167]. The three types of damages may finally induce apoptosis of follicle that reduces ovarian reserve [15].

Cigarette smoking can induce oxidative stress. Studies on disease of respiratory system have shown that cigarette smoking increased the oxidative stress markers in blood and bronchoalveolar lavage fluid [168]. Cadmium, a metal component of cigarette, was reported by the study of Nampoothiri et al. inducing ovarian oxidative stress [169]. They treated adult rats with lead acetate (LA) or cadmium acetate (CA) or both at a dose of 0.05 mg/kg body weight on a daily basis for 15 days. Both lead and cadmium can accumulate in ovarian tissue. Their results have shown that the lipid peroxides and catalase activity, both markers of oxidative stress, were increased in granulose cells of both groups, coupled with decreased superoxide dismutase activities and glutathione status, which are important roles of antioxidant system. More directly, Gannon et al. found that mice exposure to cigarette smoke for 8 weeks induced oxidative stress by increasing Hsp25 and decreasing superoxide dismutase two protein expression. At the same time, they observed a significant decrease in the number of primordial and growing follicles and the relative ovarian weight [170]. Another study conducted by Sobinoff et al. used a direct nasal exposure to cigarette smoke to mice, resulting in increased levels of primordial follicle depletion, oxidative stress, and antral follicle oocyte apoptosis, shown by increased levels of mitochondrial ROS and lipid peroxidation [171]. Camlin et al. used nasally exposed pregnant mice to cigarette for 12 weeks throughout pregnancy and lactation. Their results showed reduction in follicle numbers and elevated levels of oxidative stress in ovaries of neonatal mice. This finding indicated that maternal smoke exposure may also cause oxidative stress [172].

In addition to cigarette smoke, El-Sharkawy et al. found in animal experiment that methoxychlor (MXC) can induce oxidative stress. Methoxychlor is an organochlorine pesticide and acts as an endocrine disruptor having estrogenic, anti-androgenic, and anti-estrogenic functions in ovary. Adult female rats orally exposed to MXC of 200 mg/kg, twice/weekly, had a meaningful decrease of ovarian and body weight, decrease in progesterone levels and serum estradiol, and meaningful increase of lipid peroxidation (caused by ROS) coupled with decrease in the total antioxidant. They further observed the ovarian histopathology and found an atretic morphology in pre-antral follicle. The toxic effect of MXC was ameliorative by the administration of propolis, a substance of antioxidant [173]. However, to the best of our knowledge, the similar effects of MXC had not been reported on the other types of pesticide.

In terms of dietary habits, it was reported that the adverse effects of alcohol drinking in reproductive health may be associated with oxidative stress [174]. In contrast, β-cryptoxanthin, an antioxidant and mainly found in fruits, has shown an effect on reducing the risk of earlier onset of menopause [138].

Many kinds of food or their components show the effect of antioxidant. Ergothioneine is a natural antioxidant initially found in fungus. Natural ergothioneine can eliminate free radical and maintain the normal function of DNA. Alpha-lipoic acid is an enzyme that exists in mitochondria. With both the fat-soluble and water-soluble properties, alpha-lipoic acid can effectively eliminate the free radical in human body and is widely used against oxidative stress in many

pathological situations, such as diabetes, Parkinson disease, and cardiovascular diseases. Vitamin E, carotene, and astaxanthin were also reported to have the effect of antioxidant. In addition, some kinds of drug were reported to directly act against the oxidative effects in ovary. The signaling pathway of NF-E2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1)-antioxidant response element (ARE) defends against oxidative stress. Akino N et al. showed that dimethylfumarate can activate Nrf2/Keap1 pathway and alleviate oxidative stress in the mouse ovary [175]. Moreover, Niringiyumukiza JD et al. reported glycogen synthase kinase-3 (GSK-3) can inhibit doxorubicin-induced oxidative damage in mice via GSK-3/Nrf2 signaling pathway [176]. Alpha-lipoic acid [177], traditional Chinese medicine Kuntai capsule [178], coenzyme Q10 [179], and resveratrol were also reported effective against oxidative stress in ovary. Eliminating of oxidative stress in ovary may facilitate to maintain ovarian reserve and reduce the risk of POI.

4.4.3 Environmental and Lifestyle Factors Affect Endocrine System

Ovarian endocrine system is a complicated and elegant hormone network that controls the folliculogenesis, menstrual cycle, and fertility. The hypothalamus-pituitary-ovary axis dominates the release of sexual hormone. Hypothalamus releases the gonadotropin-releasing hormone (GnRH) in pulse, which targets the release of gonadotropin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), by pituitary. FSH supports the follicular growth and stimulates secretion of estrogen by granulocyte. Estrogen suppresses the release of hypothalamus and pituitary by GnRH or gonadotropin through the negative feedback regulation [179].

Folliculogenesis mainly depends on the interaction between anti-Müllerian hormone (AMH), FSH, androgens, and estradiol (E2) in human ovarian. Based on the development of follicle, folliculogenesis can be divided into gonadotropin-independent follicle growth period (before pre-antral follicle) and gonadotropin-dependent follicle growth period (antral follicle till ovulation). Before pre-antral follicle, folliculogenesis relies on the intra-ovarian factors, which are mainly androgens, AMH, and FSH. Androgens are important to early follicular growth. It was reported that testosterone and dihydrotestosterone (DHT) can increase the number of growing follicles as well as a greater proliferation of granulosa cells (GCs) and theca cells (TCs) in animal experiment. It is suggested that androgens exerted this effect by enhanced FSH receptor expression in pre-antral follicle. Although FSH is a gonadotropin, growing evidence has shown that FSH stimulated follicle growth moderately during the gonadotropin-independent follicle growth period synergized with other stimulating factors. AMH is a glycoprotein of the TGF-β family. Secreted by GCs, it negatively controls the initial follicular recruitment through counteracting growth-promoting effects of FSH on granulosa cells. AMH gene knockout mouse models show a meaningful increase of growing follicles at all stages. Thus, AMH is

important to activate the primordial follicle pool and its levels are treated as a marker of ovarian reserve. After pre-antral follicle period, folliculogenesis relies on gonadotropin. FSH binds in G-protein reporter then activates target gene via AC-CAMP-PKA signaling pathway on GCs. Those activated genes are important for GCs maturation, LH reporter expression, follicular survival, and estrogen secretion. Estrogen can directly support the follicular maturation, and negatively control FSH secretion, which means the prevention of follicular over activation and selection of dominant follicle. The close cooperation of those sexual hormones is responsible for normal folliculogenesis. Therefore, normal levels of androgen, estrogen, AMH, and FSH are essential for maintaining ovarian reserve [156].

Cigarette smoking, the most established risk factor of POI, can affect the effectiveness of sexual hormone, especially estrogen, through many pathways. It is well known that cigarette smoking has anti-estrogen effect, but how does the effect work? We summarize those effects in the following categories. (1) Cigarette smoking reduces expression of estrogen. It was reported that the urinary estrogen level is lower in smokers [118]. As we discussed previously, PAHs activate the dead signaling pathways of GCs and cause apoptosis [159]. Estrogen is mainly synthesized by GCs, and apoptosis of GCs can decrease the level of estrogen in serum. In addition, some component of cigarette, such as nicotine, cotinine, and anabasine, could inhibit the activity of aromatase [180]. Aromatase converts androstenedione into estradiol in GCs. (2) Cigarette smoking accelerates the elimination of estrogen. PAHs have enzyme-inducing functions, especially on the cytochrome P450 (CYP) family of enzymes. PAHs exposure results in enhanced clearance of CYP1A-metabolized drugs, which may accelerate the elimination of serum estrogen [181]. On the other hand, cigarette smoking enhances the 2-hydroxylation pathway in the liver and could convert estrogens into 2-hydroxyestrogens, which are less bio-active in peripheral tissues [182]. (3) Cigarette smoking increases the androgen level in serum that is against the effect of estrogen. The minimal activity of aromatase in smokers fails to convert androstenedione, which may cause androgen accumulation. Moreover, it is reported that the adrenal gland in smokers produced more androgen [183].

Other lifestyle factors affect folliculogenesis mainly through endocrine disorder, though the underlying mechanisms have not been fully understanding. Alcohol consumption is proved to be associated with change of FSH levels [184]; however, the direction of the association is contradictory [128]. In addition, those reports of the effect of alcohol consumption in women reproductive health were also inconsistent. Some studies reported that there was less or no adverse effect, while others reported that abstainers had higher risk of irregular and short cycles compared with women with low weekly alcohol consumption [185]. Therefore, regarding alcohol consumption, the association between change of FSH levels and its effects on ovarian functions remains unclear. The effect of caffeine intake was speculatively associated with estradiol. Both estradiol and caffeine are metabolized by CYP1A2 in liver [186]. Study found that caffeine intake was negatively related to estradiol levels; however, positive and no relation were also reported [187]. Other possible mechanism is that caffeine suppresses the activity of aromatase, and it was reported

that sex hormone Y binding globulin had a positive relationship with caffeine consumption [187, 188].

Regarding body mass, studies had reported that the serum levels of AMH were lower in obesity women, which indicated a lower ovarian reserve [189]. In contrast, adipose tissue can produce estradiol in postmenopausal women, but this effect is minimal during reproductive age. However, it speculates that when estrogen levels decrease in pre-menopause period, adipose would produce additional estradiol, which may explain that some studies observed that obesity women experienced a later onset of menopause, compared to women with normal BMI or underweight [140]. In terms of diet, protein consumption may be associated with AMH and ovarian reserve, but it needs further research. Phytoestrogens from soy production are known to be both estrogenic and anti-estrogenic, and its exact effect on ovarian function also requires further research [190]. High intensity of physical activity may affect hypothalamus-pituitary-ovary axis, suppress the effectiveness of GnRH and gonadotropin, which is supported by the fact that female athletes may experience prolonged menstrual cycle [145].

In summary, the secular hormones in ovarian, especially androgen, estrogen, AMH, and FSH are important for folliculogenesis. Lifestyle and environmental factors affecting the synthesis and secretion of these hormones may disturb possession of folliculogenesis and affect ovarian reserve. Cigarette smoking affects serum estrogen levels via several approaches. Other lifestyle factors may affect the expression of these hormones, though the underlying mechanisms need further investigation. Though substantial studies reported environmental factors, such as phthalates and pesticides, affected folliculogenesis, few studies investigated the association with these hormones.

4.4.4 Environmental and Lifestyle Factors Induce Epigenetic Modification

Epigenetic modification refers to the change in gene expression without alteration in DNA nucleotide sequence. Human and many creatures are composed of various types of cells with different phenotype, which are distinguished in cellular structure, functions, and gene expression. Even the same type of cells are variant in phenotype in different timing and space. However, given that all the cells in an individual share the same DNA sequence, the variance of phenotype seems impossible to happen and raise a contradiction. Epigenetic modification resolves this contradiction. Distinguished from transcription and translation, such genetic conception, epigenetic modification regulates gene expression via DNA methylation, histone modification, RNA interference, and so on. It guarantees specific gene expression in the right time and right place, and is reversible and can be steadily passed on to offspring [191].

It has been reported in animal studies that exposure to some environmental endocrine disruptors leads to loss of the primordial follicle pool in the offspring,

indicating that the effect of some harmful factors is transgenerational [15]. This transgenerational effect could result from genetic changes and, more likely, result from epigenetic changes. Diethylhexyl phthalate (DEHP) is an estrogen-like environmental endocrine disruptors. Zhang et al. injected DEHP to newborn female mice at doses of 20 and 40 μg/kg per body weight during the weaning period. Results have shown that newborn female mice exposure to DEHP decreased the amount of the primordial follicles in pubertal and adult age, with a reducing level of imprinted gene methylation on the oocytes [192]. Furthermore, they treated pregnant mice with DEHP at doses of 0 and 40 μg/kg body weight from 0.5 to 18.5 day post coitus. In this experiment, they found that in the F1 generations of DEHP treating mice, the percentage of methylated CpG sites in Igf2r and Peg3, which are differentially methylated regions, was reducing in primordial germ cells. Maternally methylated Igf2r and Peg3, which erased when PGCs reached genital ridges in mice, and re-established during gametogenesis, are important for germ cell functions [193]. Moreover, they surprisingly found that the modification of the DNA methylation of imprinted genes in F1 mouse oocytes was heritable to F2 generations. In their further study, they found that methylation level of another gene Stra8 was increased and the expression levels of Stra8 protein were significantly decreased in mice with maternal exposure to DEHP. Protein Stra8 is one of the steroidogenic proteins [194].

Another environmental endocrine disruptor bisphenol A (BPA) was reported to be decreasing methylated levels of several genes. Pathl et al. found that exposure to BPA resulted in cardiac epigenetic marks changes [195]. The epigenetic effects of BPA on ovary may be similar to DEHP. Zhang et al. found that material exposure to BPA resulted in decreased methylated levels of Igf2r, Peg3, and H19 in fetal mouse germ cells, which may affect gametogenesis [196].

It was reported that methoxychlor (MXC), a pesticide, affects ovarian function via altered methylation patterns. Zama AM et al. exposed rats to MXC for 100 mg/kg per day between embryonic day 19 and postnatal day 7. They found a significant hypermethylation in the ER-beta promoter regions, with increased DNA methyltransferase 3b (Dnmt3b) levels in rats postnatal day 50–60. These findings indicated that MXC causes hypermethylation in ovary via Dnmt3b and alters ovarian functions [197].

In summary, environmental endocrine disruptors, such as DEHP and BPA, and some kinds of pesticide may alter methylated level of several genes in ovary, which may affect germ cells development or sexual hormone activity. However, further researches are required in this field.

4.4.5 Environmental and Lifestyle Factors Cause DNA Damage in Ovary

DNA damage refers to the permanent changes in DNA nucleotide sequence, and subsequently leads to apoptosis, function changes or loss, and change in genotype. The types of DNA damage included nucleotide point mutation, deletion, insertion, and DNA chains transposition or fracture. Various physical or chemical factors can cause DNA damage. Causing DNA damage is also a notable approach that leads to ovarian function loss [198].

Cigarette contains hundreds of components making it quite easy to cause DNA damage. BaP–DNA adducts have been detected in human luteal cell. Moreover, in smoking women who underwent IVF, researchers observed higher rates of DNA strand breaks. Study in mouse and bovine found cigarette components interfered meiosis of oocyte. Mouse models exposed to nicotine resulted in premature separation of sister chromatids and chromosomal abnormalities during meiosis. Moreover, the exposure to nicotine also resulted in an increased rate of aneuploidy, abnormal chromosomal alignment, multipolar and malformed spindles, and disorganized microfilaments in the bovine oocytes. And BaP exposure may cause meiotic spindle disturbances. These changes in DNA may lead to oocyte apoptosis and follicular atresia [156].

Radiation is well known to cause DNA damage, and exposure to ultraviolet ray increases the odds of skin cancer via DNA damage. It was reported that exposure to mobile phone radiation could increase the expression of ATM, a DNA damage repair protein, as previously discussed, which indicated that radiant factors may cause DNA damage. In addition, ROS can attack DNA chains and lead to DNA damage, moreover, factors can cause oxidative stress, thus, can be risk factors of DNA damage.

4.4.6 Lifestyle and Environmental Factors Affect the Cell-To-Cell Communication Between Oocyte and Other Cells

Throughout the whole folliculogenesis, oocyte meets different cells in different period. During the process of PGC formation and specification, the bone morphogenetic protein (BMP) family signaling from the surrounding extraembryonic ectoderm (ExE) and visceral endoderm (VE) is extremely important. After arriving at genital ridges, developmental direction of the bipotential PGCs is determined by the somatic supporting cells in the gonad. During follicular growing period, oocyte is surrounded in an enclosed space by theca cells and GCs. The communications between oocyte and its surrounded cells are essential for folliculogenesis and various in ways. Endocrine communication may be the dominant way; however, the effect of

cell-to-cell communication should not be ignored, especially between oocyte and GCs [154].

GCs used to be a flattened epithelium surrounding the oocyte. As follicle develops, GCs become cuboidal and form multiple layers. During antral follicle period, as the expansion of antrum in follicle, GC divides into two parts. GCs that separate away from oocyte were called mural GC that lines the follicle wall. The other adjacent to the oocyte is called cumulus GC. The mural GCs are major source for steroid hormones. In this view, follicle can be treated as a functional syncytium comprised by oocyte in the center, cumulus GCs around the oocyte, mural GCs in the follicular walls, and the gap junctions connecting these cells [157, 199].

Gap junctions are constituted of intercellular channels that directly connect adjacent cells and allow the diffusional movement of ions, metabolites, and other potential signaling molecules [199]. The gap junctions contain several different connexins, a family of more than 13 related proteins, such as connexins 32, 43, and 45. Ackert CL et al. assessed the importance of connexins 43 gap junctions. They grafted ovaries of late gestation mouse fetuses or newborn pups who are lacking connexin 43 into the kidney capsules of adult females, then allowing them to develop for up to 3 weeks, resulting follicles failed to develop beyond the primary stage. At the same time, the morphology of those mutant follicles was abnormal, meanwhile the zona pellucida was poorly developed, moreover the cytoplasm of both granulosa cells and oocytes was vacuolated, and cortical granules were absent from the oocytes [200]. Another article found connexin 37-deficient mice lacked mature (Graafian) follicles, and failed to ovulate and develop numerous inappropriate corpora luteum [199]. These findings indicate that cell-to-cell communications through gap junctions are important for folliculogenesis.

Several studies indicated that lifestyle and environmental factors may potentially affect folliculogenesis through interfering the functions of gap junctions in ovary. Paksy K et al. cultured human ovarian follicular with cadmium (Cd) and resulted in morphological changes in GCs, interfered with cell–cell junctions and the adherence of cells [201]. Sharovskaya J et al. cultured hepatoma cell with carcinogenic or non-carcinogenic PAHs, and they found that the carcinogenic PAHs can affect the formation of gap junctions in hepatoma and interfere gap junction intercellular communication directly [202]. One study on testis Sertoli cells found that several testicular toxicants, including cadmium chloride, bisphenol A, and pesticides affected intercellular junctions through reducing the level of connexins 43 [203]. However, lack of direct evidences from ovarian studies suggests that the association between environmental or lifestyles factors and gap junction intercellular communication in ovary needs further research to clarify.

4.5 Conclusions and Suggestions

In summary, the model of modern society has been greatly changed, including their living environment and lifestyle. The changes of natural environmental factors, social environmental factors, and lifestyle factors may cause positive or negative effects on women reproductive health, however, the negative parts are usually greater. Thus, we should pay more attention to these factors and find out when and how they jeopardize women's health, and finally reduce or prevent the harmful effects of those factors.

However, considerable works must be finished, given that there remains controversial and inexplicable findings found in those literature. In addition, most of these studies only discussed the influence on folliculogenesis and reduced ovarian reserve, which was just one of the risk factors of POI. Most of the papers we discuss were based on animal experiments and not directly involving POI. Some of these harmful factors remain poorly understood. Therefore, further investigations are required.

As the development of systems medicine, doctors and scientists realize that health care is not only about treatment of diseases itself, it should also be able to promote human physical and mental health, and allow every person to enjoy their daily life and work effective with a healthy body. Therefore, prevention of disease becomes quite important. A better learning and understanding about the relationship between women ovarian function and the outside environmental factors will provide women more information to facilitate their reproductive health.

References

1. Jankowska K. Premature ovarian failure. Prz Menopauzalny. 2017;16(2):51–6.
2. European Society for Human Reproduction and Embryology, Embryology Guideline Group on POI, Webber L, et al. ESHRE guideline: management of women with premature ovarian insufficiency. Hum Reprod. 2016;31(5):926–37.
3. 吴结英, 胡卫华. <卵巢早衰的病因学研究进展_吴结英 (1). 2019.
4. Nippita TA, Baber RJ. Premature ovarian failure: a review. Climacteric. 2007;10(1):11–22.
5. Richardson MC, Guo M, Fauser BC, et al. Environmental and developmental origins of ovarian reserve. Hum Reprod Update. 2014;20(3):353–69.
6. Ge W, Li L, Dyce PW, et al. Establishment and depletion of the ovarian reserve: physiology and impact of environmental chemicals. Cell Mol Life Sci. 2019;76(9):1729–46.
7. Haruty B, Friedman J, Hopp S, et al. Reproductive health and the environment: counseling patients about risks. Cleve Clin J Med. 2016;83(5):367–72.
8. Le Cann P, Bonvallot N, Glorennec P, et al. Indoor environment and children's health: recent developments in chemical, biological, physical and social aspects. Int J Hyg Environ Health. 2011;215(1):1–18.
9. Barnes SK, Ozanne SE. Pathways linking the early environment to long-term health and lifespan. Prog Biophys Mol Biol. 2011;106(1):323–36.
10. Gascon M, Vrijheid M, Nieuwenhuijsen MJ. The built environment and child health: an overview of current evidence. Curr Environ Health Rep. 2016;3(3):250–7.
11. Bergman A, Heindel JJ, Kasten T, et al. The impact of endocrine disruption: a consensus statement on the state of the science. Environ Health Perspect. 2013;121(4):A104–6.

12. Nomiri S, Hoshyar R, Ambrosino C, et al. A mini review of bisphenol A (BPA) effects on cancer-related cellular signaling pathways. Environ Sci Pollut Res Int. 2019;26(9):8459–67.
13. Fernandez SV, Huang Y, Snider KE, et al. Expression and DNA methylation changes in human breast epithelial cells after bisphenol A exposure. Int J Oncol. 2012;41(1):369–77.
14. de Araujo JFP, Podratz PL, Merlo E, et al. Organotin exposure and vertebrate reproduction: a review. Front Endocrinol (Lausanne). 2018;9:64.
15. Vabre P, Gatimel N, Moreau J, et al. Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. Environ Health. 2017;16(1):37.
16. Lee JE, Jung HW, Lee YJ, et al. Early-life exposure to endocrine-disrupting chemicals and pubertal development in girls. Ann Pediatr Endocrinol Metab. 2019;24(2):78–91.
17. Rattan S, Zhou C, Chiang C, Mahalingam S, Brehm E, Flaws JA. Exposure to endocrine disruptors during adulthood: consequences for female fertility. J Endocrinol. 2017;233(3): R109–29.
18. Rattan S, Flaws JA. The epigenetic impacts of endocrine disruptors on female reproduction across generationsdagger. Biol Reprod. 2019;101(3):635–44.
19. Net S, Sempéré R, Delmont A, et al. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environ Sci Technol. 2015;49(7):4019–35.
20. Berge A, Cladiere M, Gasperi J, et al. Meta-analysis of environmental contamination by phthalates. Environ Sci Pollut Res Int. 2013;20(11):8057–76.
21. Xu C, Chen JA, Qiu Z, et al. Ovotoxicity and PPAR-mediated aromatase downregulation in female Sprague-Dawley rats following combined oral exposure to benzo[a]pyrene and di-(2-ethylhexyl) phthalate. Toxicol Lett. 2010;199(3):323–32.
22. Li L, Liu JC, Lai FN, et al. Di (2-ethylhexyl) phthalate exposure impairs growth of antral follicle in mice. PLoS One. 2016;11(2):e0148350.
23. Hannon PR, Niermann S, Flaws JA. Acute exposure to Di(2-Ethylhexyl) phthalate in adulthood causes adverse reproductive outcomes later in life and accelerates reproductive aging in female mice. Toxicol Sci. 2016;150(1):97–108.
24. Hannon PR, Flaws JA. The effects of phthalates on the ovary. Front Endocrinol (Lausanne). 2015;6:8.
25. Moyer B, Hixon ML. Reproductive effects in F1 adult females exposed in utero to moderate to high doses of mono-2-ethylhexylphthalate (MEHP). Reprod Toxicol. 2012;34(1):43–50.
26. Zhang Y, Mu X, Gao R, et al. Foetal-neonatal exposure of Di (2-ethylhexyl) phthalate disrupts ovarian development in mice by inducing autophagy. J Hazard Mater. 2018;358:101–12.
27. Liu JC, Lai FN, Li L, et al. Di (2-ethylhexyl) phthalate exposure impairs meiotic progression and DNA damage repair in fetal mouse oocytes in vitro. Cell Death Dis. 2017;8(8):e2966.
28. Muczynski V, Lecureuil C, Messiaen S, Guerquin MJ, N'Tumba-Byn T, Moison D, Hodroj W, Benjelloun H, Baijer J, Livera G, Frydman R. Cellular and molecular effect of MEHP involving LXRα in human fetal testis and ovary. PLoS One. 2012;7(10):e48266.
29. Zhang JN, Zhang RQ, Liu JC, et al. Di (2-ethylhexyl) phthalate exposure impairs the microRNAs expression profile during primordial follicle assembly. Front Endocrinol (Lausanne). 2019;10:877.
30. Ritter R, Scheringer M, MacLeod M, Moeckel C, Jones KC, Hungerbühler K. Intrinsic human elimination half-lives of polychlorinated biphenyls derived from the temporal evolution of cross-sectional biomonitoring data from the United Kingdom. Environ Health Perspect. 2011;119(2):225–31.
31. Vorkamp K. An overlooked environmental issue? A review of the inadvertent formation of PCB-11 and other PCB congeners and their occurrence in consumer products and in the environment. Sci Total Environ. 2016;541:1463–76.
32. Kezios KL, Liu X, Cirillio PM, et al. Prenatal polychlorinated biphenyl exposure is associated with decreased gestational length but not birth weight: archived samples from the child health and development studies pregnancy cohort. Environ Health. 2012;11:49.

33. Murati T, Simic B, Brozovic A, et al. PCB 77 action in ovary cells--toxic effects, apoptosis induction and cell cycle analysis. Toxicol Mech Methods. 2015;25(4):302–11.
34. Shirota M, Mukai M, Sakurada Y, Doyama A, Inoue K, Haishima A, Akahori F, Shirota K. Effects of vertically transferred 3, 3′, 4, 4′, 5-pentachlorobiphenyl (PCB-126) on the reproductive development of female rats. J Reprod Dev. 2006;52(6):751–61.
35. Gallo MV, Ravenscroft J, Carpenter DO, et al. Persistent organic pollutants as predictors of increased FSH:LH ratio in naturally cycling, reproductive age women. Environ Res. 2018;164:556–64.
36. Lorber M, Schecter A, Paepke O, et al. Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. Environ Int. 2015;77:55–62.
37. Rowell C, Kuiper N, Preud'Homme H. Is container type the biggest predictor of trace element and BPA leaching from drinking water bottles? Food Chem. 2016;202:88–93.
38. Sogorb MA, Estevez J, Vilanova E. Case study: is bisphenol S safer than bisphenol A in thermal papers? Arch Toxicol. 2019;93(7):1835–52.
39. Vervliet P, de Nys S, Boonen I, et al. Qualitative analysis of dental material ingredients, composite resins and sealants using liquid chromatography coupled to quadrupole time of flight mass spectrometry. J Chromatogr A. 2018;1576:90–100.
40. Alkasir RS, Rossner A, Andreescu S. Portable colorimetric paper-based biosensing device for the assessment of Bisphenol a in indoor dust. Environ Sci Technol. 2015;49(16):9889–97.
41. Toner F, Allan G, Dimond SS, et al. In vitro percutaneous absorption and metabolism of bisphenol A (BPA) through fresh human skin. Toxicol In Vitro. 2018;47:147–55.
42. Grimaldi M, Boulahtouf A, Toporova L, et al. Functional profiling of bisphenols for nuclear receptors. Toxicology. 2019;420:39–45.
43. Moreman J, Lee O, Trznadel M, et al. Acute toxicity, Teratogenic, and estrogenic effects of Bisphenol a and its alternative replacements bisphenol S, bisphenol F, and bisphenol AF in Zebrafish embryo-larvae. Environ Sci Technol. 2017;51(21):12796–805.
44. Huang X, Cang X, Liu J. Molecular mechanism of Bisphenol a on androgen receptor antagonism. Toxicol In Vitro. 2019;61:104621.
45. Chen Y, Wang Y, Ding G, et al. Association between bisphenol a exposure and idiopathic central precocious puberty (ICPP) among school-aged girls in Shanghai, China. Environ Int. 2018;115:410–6.
46. Hass U, Christiansen S, Boberg J, et al. Low-dose effect of developmental bisphenol a exposure on sperm count and behaviour in rats. Andrology. 2016;4(4):594–607.
47. Signorile PG, Spugnini EP, Citro G, Viceconte R, Vincenzi B, Baldi F, Baldi A. Endocrine disruptors in utero cause ovarian damages linked to endometriosis. Front Biosci (Elite Ed). 2012;4:1724–30.
48. Zhang HQ, Zhang XF, Zhang LJ, Chao HH, Pan B, Feng YM, Li L, Sun XF, Shen W. Fetal exposure to bisphenol A affects the primordial follicle formation by inhibiting the meiotic progression of oocytes. Mol Biol Rep. 2012;39(5):5651–7.
49. Qiu J, Sun Y, Sun W, Wang Y, Fan T, Yu J. Neonatal exposure to bisphenol A advances pubertal development in female rats. Mol Reprod Dev. 2020;87(4):503–11.
50. Newbold RR, Jefferson WN, Padilla-Banks E. Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. Reprod Toxicol. 2007;24(2):253–8.
51. Kandaraki E, Chatzigeorgiou A, Livadas S, et al. Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. J Clin Endocrinol Metab. 2011;96(3):E480–4.
52. 吴一华2, 夏大静. <环境内分泌干扰物的女性生殖毒性及其在妇科肿瘤发生发展中.pdf>. 2019.
53. Practice Committee of American Society for Reproductive Medicine. Smoking and infertility. Fertil Steril. 2008;90(5 Suppl):S254–9.
54. Practice Committee of the American Society for Reproductive Medicine. Smoking and infertility: a committee opinion. Fertil Steril. 2012;98(6):1400–6.

55. Practice Committee of the American Society for Reproductive Medicine. Smoking and infertility: a committee opinion. Fertil Steril. 2018;110(4):611–8.
56. Kilic S, Yuksel B, Lortlar N, et al. Environmental tobacco smoke exposure during intrauterine period promotes granulosa cell apoptosis: a prospective, randomized study. J Matern Fetal Neonatal Med. 2012;25(10):1904–8.
57. Paixão LL, Gaspar-Reis RP, Gonzalez GP, Santos AS, Santana AC, Santos RM, Spritzer PM, Nascimento-Saba CC. Cigarette smoke impairs granulosa cell proliferation and oocyte growth after exposure cessation in young Swiss mice: an experimental study. J Ovarian Res. 2012;5 (1):25.
58. Wesselink AK, Hatch EE, Rothman KJ, et al. Prospective study of cigarette smoking and fecundability. Hum Reprod. 2019;34(3):558–9.
59. Hull MG, North K, Taylor H, Farrow A, Ford WC. Delayed conception and active and passive smoking. Fertil Steril. 2000;74(4):725–33.
60. Wang P, Tian Y, Wang XJ, et al. Organophosphate pesticide exposure and perinatal outcomes in Shanghai. China Environ Int. 2012;42:100–4.
61. Ding G, Cui C, Chen L, et al. Prenatal exposure to pyrethroid insecticides and birth outcomes in rural northern China. J Expo Sci Environ Epidemiol. 2015;25(3):264–70.
62. Hu Y, Ji L, Zhang Y, et al. Organophosphate and pyrethroid pesticide exposures measured before conception and associations with time to pregnancy in Chinese couples enrolled in the Shanghai birth cohort. Environ Health Perspect. 2018;126(7):077001.
63. Fei J, Qu JH, Ding XL, et al. Fenvalerate inhibits the growth of primary cultured rat preantral ovarian follicles. Toxicology. 2010;267(1–3):1–6.
64. Guerra MT, de Toledo FC, Kempinas WG. In utero and lactational exposure to fenvalerate disrupts reproductive function in female rats. Reprod Toxicol. 2011;32(3):298–303.
65. Rao RP, Kaliwal BB. Monocrotophos induced dysfunction on estrous cycle and follicular development in mice. Ind Health. 2002;40(3):237–44.
66. Tello JA, Kohout T, Pineda R, et al. Reproductive physiology of a humanized GnRH receptor mouse model: application in evaluation of human-specific analogs. Am J Physiol Endocrinol Metab. 2013;305(1):E67–77.
67. Nanda N, Kaliwal BB. Effect of edifenphos on compensatory ovarian hypertrophy, follicular kinetics and estrous cycle in hemicastrated rats. J Basic Clin Physiol Pharmacol. 2003;14 (4):373–86.
68. Mahadevaswami MP, Kaliwal BB. Effect of dimethoate administration schedules on compensatory ovarian hypertrophy, follicular dynamics, and estrous cycle in hemicastrated mice. J Basic Clin Physiol Pharmacol. 2002;13(3):225–48.
69. Farr SL, Cooper GS, Cai J, et al. Pesticide use and menstrual cycle characteristics among premenopausal women in the Agricultural Health Study. Am J Epidemiol. 2004;160 (12):1194–204.
70. Zhang Y, Li Z, Gao Y, et al. Effects of fetal microwave radiation exposure on offspring behavior in mice. J Radiat Res. 2015;56(2):261–8.
71. 徐少强　马胡.　<微波辐射对男性生殖系统及生殖内分泌影响的META分析_马春晓.pdf>. 2018.
72. Adriaens I, Smitz J, Jacquet P. The current knowledge on radiosensitivity of ovarian follicle development stages. Hum Reprod Update. 2009;15(3):359–77.
73. Gosden RG, Wade JC, Fraser HM, Sandow J, Faddy MJ. Impact of congenital or experimental hypogonadotrophism on the radiation sensitivity of the mouse ovary. Hum Reprod. 1997;12 (11):2483–8.
74. 胡凌云. <X线辐射对大鼠卵巢形态与功能的影响_胡凌云.pdf>. 2011.
75. Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update. 2001;7(6):535–43.
76. Panagopoulos DJ. Effect of microwave exposure on the ovarian development of Drosophila melanogaster. Cell Biochem Biophys. 2012;63(2):121–32.

77. Gul A, Celebi H, Ugras S. The effects of microwave emitted by cellular phones on ovarian follicles in rats. Arch Gynecol Obstet. 2009;280(5):729–33.
78. Diem E, Schwarz C, Adlkofer F, et al. Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. Mutat Res. 2005;583(2):178–83.
79. 罗亚萍 马陈. <拟手机辐射对大鼠卵巢功能和卵巢ATM蛋白表达的影响_马惠荣.pdf>. 2014.
80. Jensen K, Hahn NE, Palme R, Saxton K, Francis DD. Vacuum-cleaner noise and acute stress responses in female C57BL/6 mice (Mus musculus). J Am Assoc Lab Anim Sci. 2010;49 (3):300–6.
81. Takahashi M. Heat stress on reproductive function and fertility in mammals. Reprod Med Biol. 2012;11(1):37–47.
82. Das R, Sailo L, Verma N, et al. Impact of heat stress on health and performance of dairy animals: a review. Vet World. 2016;9(3):260–8.
83. Wakayo BU, Brar PS, Prabhakar S. Review on mechanisms of dairy summer infertility and implications for hormonal intervention. Open Vet J. 2015;5(1):6–10.
84. Su YQ, Wu X, O'Brien MJ, et al. Synergistic roles of BMP15 and GDF9 in the development and function of the oocyte-cumulus cell complex in mice: genetic evidence for an oocyte-granulosa cell regulatory loop. Dev Biol. 2004;276(1):64–73.
85. Voronina E, Lovasco LA, Gyuris A, et al. Ovarian granulosa cell survival and proliferation requires the gonad-selective TFIID subunit TAF4b. Dev Biol. 2007;303(2):715–26.
86. Da Silva-Buttkus P, Jayasooriya GS, Mora JM, et al. Effect of cell shape and packing density on granulosa cell proliferation and formation of multiple layers during early follicle development in the ovary. J Cell Sci. 2008;121(Pt 23):3890–900.
87. Li L, Wu J, Luo M, et al. The effect of heat stress on gene expression, synthesis of steroids, and apoptosis in bovine granulosa cells. Cell Stress Chaperones. 2016;21(3):467–75.
88. Alemu TW, Pandey HO, Salilew Wondim D, et al. Oxidative and endoplasmic reticulum stress defense mechanisms of bovine granulosa cells exposed to heat stress. Theriogenology. 2018;110:130–41.
89. Luo M, Li L, Xiao C, et al. Heat stress impairs mice granulosa cell function by diminishing steroids production and inducing apoptosis. Mol Cell Biochem. 2016;412(1–2):81–90.
90. Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact. 2014;224:164–75.
91. Kaczmarek M. The timing of natural menopause in Poland and associated factors. Maturitas. 2007;57(2):139–53.
92. Canavez FS, Werneck GL, Parente RC, et al. The association between educational level and age at the menopause: a systematic review. Arch Gynecol Obstet. 2011;283(1):83–90.
93. Liberatos P, Link BG, Kelsey JL. The measurement of social class in epidemiology. Epidemiol Rev. 1988;10(1):87–121.
94. Dorjgochoo T, Kallianpur A, Gao YT, et al. Dietary and lifestyle predictors of age at natural menopause and reproductive span in the Shanghai Women's Health Study. Menopause. 2008;15(5):924–33.
95. Marmot M, Wilkinson RG. Psychosocial and material pathways in the relation between income and health: a response to Lynch et al. BMJ. 2001;322(7296):1233–6.
96. Brett KM, Cooper GS. Associations with menopause and menopausal transition in a nationally representative US sample. Maturitas. 2003;45(2):89–97.
97. Carda SN, Bilge SA, Öztürk TN, Oya G, Ece O, Hamiyet B. The menopausal age, related factors and climacteric symptoms in Turkish women. Maturitas. 1998;30(1):37–40.
98. Nagata C, Takatsuka N, Kawakami N, Shimizu H. Association of diet with the onset of menopause in Japanese women. Am J Epidemiol. 2000;152(9):863–7.
99. Ozdemir O, Col M. The age at menopause and associated factors at the health center area in Ankara, Turkey. Maturitas. 2004;49(3):211–9.

100. Wise LA, Krieger N, Zierler S, Harlow BL. Lifetime socioeconomic position in relation to onset of perimenopause. J Epidemiol Community Health. 2002;56(11):851–60.
101. Barthold JA, Myrskylä M, Jones OR. Childlessness drives the sex difference in the association between income and reproductive success of modern Europeans. Evol Hum Behav. 2012;33 (6):628–38.
102. Krzyzanowska M, Mascie-Taylor CG. Educational and social class assortative mating in fertile British couples. Ann Hum Biol. 2014;41(6):561–7.
103. Tianzhu Z, Shihai Y, Juan D. Antidepressant-like effects of cordycepin in a mice model of chronic unpredictable mild stress. Evid Based Complement Alternat Med. 2014;2014:438506.
104. Tseng LA, El Khoudary SR, Young EA, et al. The association of menopause status with physical function: the Study of Women's Health Across the Nation. Menopause. 2012;19 (11):1186–92.
105. Allshouse AA, Semple AL, Santoro NF. Evidence for prolonged and unique amenorrhea-related symptoms in women with premature ovarian failure/primary ovarian insufficiency. Menopause. 2015;22(2):166–74.
106. Fu XY, Chen HH, Zhang N, et al. Effects of chronic unpredictable mild stress on ovarian reserve in female rats: feasibility analysis of a rat model of premature ovarian failure. Mol Med Rep. 2018;18(1):532–40.
107. Barra R, Cruz G, Mayerhofer A, et al. Maternal sympathetic stress impairs follicular development and puberty of the offspring. Reproduction. 2014;148(2):137–45.
108. Dorfman M, Arancibia S, Fiedler JL, et al. Chronic intermittent cold stress activates ovarian sympathetic nerves and modifies ovarian follicular development in the rat. Biol Reprod. 2003;68(6):2038–43.
109. Dechanet C, Anahory T, Mathieu Daude JC, et al. Effects of cigarette smoking on reproduction. Hum Reprod Update. 2010;17(1):76–95.
110. Schoenaker DAJM, Jackson CA, Rowlands JV, et al. Socioeconomic position, lifestyle factors and age at natural menopause: a systematic review and meta-analyses of studies across six continents. Int J Epidemiol. 2014;43(5):1542–62.
111. Zhu D, Chung HF, Pandeya N, et al. Relationships between intensity, duration, cumulative dose, and timing of smoking with age at menopause: a pooled analysis of individual data from 17 observational studies. PLoS Med. 2018;15(11):e1002704.
112. van Asselt KM, Kok HS, van der Schouw YT, et al. Current smoking at menopause rather than duration determines the onset of natural menopause. Epidemiology. 2004;15(5):634–9.
113. Hayatbakhsh MR, Clavarino A, Williams GM, et al. Cigarette smoking and age of menopause: a large prospective study. Maturitas. 2012;72(4):346–52.
114. Hyland A, Piazza K, Hovey KM, et al. Associations between lifetime tobacco exposure with infertility and age at natural menopause: the Women's Health Initiative Observational Study. Tob Control. 2016;25(6):706–14.
115. Gold EB, Bromberger J, Crawford S, et al. Factors associated with age at natural menopause in a multiethnic sample of midlife women. Am J Epidemiol. 2001;153(9):865–74.
116. Yang HJ, Suh PS, Kim SJ, et al. Effects of smoking on menopausal age: results from the Korea National Health and Nutrition Examination Survey, 2007 to 2012. J Prev Med Public Health. 2015;48(4):216–24.
117. Cramer DW, Harlow BL, Xu H, et al. Cross-sectional and case-controlled analyses of the association between smoking and early menopause. Maturitas. 1995;22(2):79–87.
118. Parente RC, Faerstein E, Celeste RK, et al. The relationship between smoking and age at the menopause: a systematic review. Maturitas. 2008;61(4):287–98.
119. 张燕燕, 王思凌, 李志新, et al. 2016–2017年四川省成人烟草流行现况调查. 预防医学情报杂志. 2019;35(06):581–6.
120. Cooper GS, Sandler DP, Bohlig M. Active and passive smoking and the occurrence of natural menopause. Epidemiology. 1999;10(6):771–3.

121. Mikkelsen TF, Graff-Iversen S, Sundby J, et al. Early menopause, association with tobacco smoking, coffee consumption and other lifestyle factors: a cross-sectional study. BMC Public Health. 2007;7(1):149.
122. Pokoradi AJ, Iversen L, Hannaford PC. Factors associated with age of onset and type of menopause in a cohort of UK women. Am J Obstet Gynecol. 2011;205(1):34.e1–e13.
123. Fleming LE, Levis S, LeBlanc WG, et al. Earlier age at menopause, work, and tobacco smoke exposure. Menopause. 2008;15(6):1103–8.
124. Honorato TC, Haadsma ML, Land JA, et al. In-utero cigarette smoke exposure and the risk of earlier menopause. Menopause. 2018;25(1):54–61.
125. Torgerson DJ, Thomas RE, Campbell MK, et al. Alcohol consumption and age of maternal menopause are associated with menopause onset. Maturitas. 1997;26(1):21–5.
126. Milic J, Glisic M, Voortman T, et al. Menopause, ageing, and alcohol use disorders in women. Maturitas. 2018;111:100–9.
127. Kinney A, Kline J, Levin B. Alcohol, caffeine and smoking in relation to age at menopause. Maturitas. 2006;54(1):27–38.
128. Taneri PE, Kiefte-de Jong JC, Bramer WM, et al. Association of alcohol consumption with the onset of natural menopause: a systematic review and meta-analysis. Hum Reprod Update. 2016;22(4):516–28.
129. Choi JI, K-d H, Lee DW, et al. Relationship between alcohol consumption and age at menopause: the Korea National Health and Nutrition Examination Survey. Taiwan J Obstet Gynecol. 2017;56(4):482–6.
130. Kline J, Tang A, Levin B. Smoking, alcohol and caffeine in relation to two hormonal indicators of ovarian age during the reproductive years. Maturitas. 2016;92:115–22.
131. Kinney A, Kline J, Kelly A, et al. Smoking, alcohol and caffeine in relation to ovarian age during the reproductive years. Hum Reprod. 2007;22(4):1175–85.
132. Faubion SS, Sood R, Thielen JM, et al. Caffeine and menopausal symptoms. Menopause. 2015;22(2):155–8.
133. Group ECW. Nutrition and reproduction in women. Hum Reprod Update. 2006;12 (3):193–207.
134. Sapre S, Thakur R. Lifestyle and dietary factors determine age at natural menopause. J Mid-life Health. 2014;5(1):3–5.
135. Martin LJ, Greenberg CV, Kriukov V, et al. Intervention with a low-fat, high-carbohydrate diet does not influence the timing of menopause. Am J Clin Nutr. 2006;84(4):920–8.
136. 陈慧，程冉，许良智. 卵巢早衰与膳食营养相关研究. 四川大学学报(医学版). 2017;48 (04):575–8.
137. Pearce K, Tremellen K. Influence of nutrition on the decline of ovarian reserve and subsequent onset of natural menopause. Hum Fertil (Camb). 2016;19(3):173–9.
138. Wang M, Gong WW, Hu RY, et al. Age at natural menopause and associated factors in adult women: findings from the China Kadoorie Biobank study in Zhejiang rural area. PLoS One. 2018;13(4):e0195658.
139. Nagata C, Takatsuka N, Inaba S, et al. Association of diet and other lifestyle with onset of menopause in Japanese women. Maturitas. 1998;29(2):105–13.
140. Tao X, Jiang A, Yin L, et al. Body mass index and age at natural menopause: a meta-analysis. Menopause. 2015;22(4):469–74.
141. Szegda KL, Whitcomb BW, Purdue-Smithe AC, et al. Adult adiposity and risk of early menopause. Hum Reprod. 2017;32(12):2522–31.
142. Bromberger JT, Matthews KA, Kuller LH, et al. Prospective study of the determinants of age at menopause. Am J Epidemiol. 1997;145(2):124–33.
143. Gold EB. The timing of the age at which natural menopause occurs. Obstet Gynecol Clin N Am. 2011;38(3):425–40.
144. Dratva J, Gomez Real F, Schindler C, et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. Menopause. 2009;16(2):385–94.

145. Nagata C, Wada K, Nakamura K, et al. Associations of physical activity and diet with the onset of menopause in Japanese women. Menopause. 2012;19(1):75–81.
146. Morris DH, Jones ME, Schoemaker MJ, et al. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. Am J Epidemiol. 2012;175(10):998–1005.
147. Zhao M, Whitcomb BW, Purdue-Smithe AC, et al. Physical activity is not related to risk of early menopause in a large prospective study. Hum Reprod. 2018;33(10):1960–7.
148. Costanian C, McCague H, Tamim H. Age at natural menopause and its associated factors in Canada: cross-sectional analyses from the Canadian Longitudinal Study on Aging. Menopause. 2018;25(3):265–72.
149. Luborsky JL, Meyer P, Sowers MF, et al. Premature menopause in a multi-ethnic population study of the menopause transition. Hum Reprod. 2003;18(1):199–206.
150. Sowers MF, Zheng H, Kravitz HM, et al. Sex steroid hormone profiles are related to sleep measures from polysomnography and the Pittsburgh Sleep Quality Index. Sleep. 2008;31 (10):1339–49.
151. Gold EB, Crawford SL, Avis NE, et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. Am J Epidemiol. 2013;178(1):70–83.
152. Auffray C, Chen Z, Hood L. Systems medicine: the future of medical genomics and healthcare. Genome Med. 2009;1(1):2.
153. Webber L, Davies M, Anderson R, et al. ESHRE Guideline: management of women with premature ovarian insufficiency. Hum Reprod. 2016;31(5):926–37.
154. Rimon-Dahari N, Yerushalmi-Heinemann L, Alyagor L, et al. Ovarian Folliculogenesis. Results Probl Cell Differ. 2016;58:167–90.
155. Dewailly D, Robin G, Peigne M, et al. Interactions between androgens, FSH, anti-Mullerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. Hum Reprod Update. 2016;22(6):709–24.
156. Dechanet C, Anahory T, Mathieu Daude JC, et al. Effects of cigarette smoking on reproduction. Hum Reprod Update. 2011;17(1):76–95.
157. Jurisicova A, Taniuchi A, Li H, et al. Maternal exposure to polycyclic aromatic hydrocarbons diminishes murine ovarian reserve via induction of Harakiri. J Clin Invest. 2007;117 (12):3971–8.
158. Korsh J, Shen A, Aliano K, et al. Polycyclic aromatic hydrocarbons and breast cancer: a review of the literature. Breast Care (Basel, Switz). 2015;10(5):316–8.
159. Matikainen T, Perez GI, Jurisicova A, et al. Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nat Genet. 2001;28(4):355–60.
160. Mattison DR, Nightingale MR. The biochemical and genetic characteristics of murine ovarian aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity and its relationship to primordial oocyte destruction by polycyclic aromatic hydrocarbons. Toxicol Appl Pharmacol. 1980;56 (3):399–408.
161. Tuttle AM, Stampfli M, Foster WG. Cigarette smoke causes follicle loss in mice ovaries at concentrations representative of human exposure. Hum Reprod. 2009;24(6):1452–9.
162. Sen N, Liu X, Craig ZR. Short term exposure to di-n-butyl phthalate (DBP) disrupts ovarian function in young CD-1 mice. Reprod Toxicol (Elmsford, NY). 2015;53:15–22.
163. Park S, Kim S, Jin H, et al. Impaired development of female mouse offspring maternally exposed to simazine. Environ Toxicol Pharmacol. 2014;38(3):845–51.
164. Wang W, Sun Y, Liu J, et al. Soy isoflavones administered to rats from weaning until sexual maturity affect ovarian follicle development by inducing apoptosis. Food Chem Toxicol. 2014;72:51–60.
165. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180–3.
166. Sinha N, Dabla PK. Oxidative stress and antioxidants in hypertension-a current review. Curr Hypertens Rev. 2015;11(2):132–42.
167. Luderer U. Ovarian toxicity from reactive oxygen species. Vitam Horm. 2014;94:99–127.

168. van der Vaart H, Postma DS, Timens W, et al. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. Thorax. 2004;59(8):713–21.
169. Nampoothiri LP, Agarwal A, Gupta S. Effect of co-exposure to lead and cadmium on antioxidant status in rat ovarian granulose cells. Arch Toxicol. 2007;81(3):145–50.
170. Gannon AM, Stampfli MR, Foster WG. Cigarette smoke exposure leads to follicle loss via an alternative ovarian cell death pathway in a mouse model. Toxicol Sci. 2012;125(1):274–84.
171. Sobinoff AP, Beckett EL, Jarnicki AG, et al. Scrambled and fried: cigarette smoke exposure causes antral follicle destruction and oocyte dysfunction through oxidative stress. Toxicol Appl Pharmacol. 2013;271(2):156–67.
172. Camlin NJ, Sobinoff AP, Sutherland JM, et al. Maternal smoke exposure impairs the long-term fertility of female offspring in a murine model. Biol Reprod. 2016;94(2):39.
173. El-Sharkawy EE, Kames AO, Sayed SM, et al. The ameliorative effect of propolis against methoxychlor induced ovarian toxicity in rat. Exp Toxicol Pathol. 2014;66(9–10):415–21.
174. Faut M, Rodriguez de Castro C, Bietto FM, et al. Metabolism of ethanol to acetaldehyde and increased susceptibility to oxidative stress could play a role in the ovarian tissue cell injury promoted by alcohol drinking. Toxicol Ind Health. 2009;25(8):525–38.
175. Akino N, Wada-Hiraike O, Isono W, et al. Activation of Nrf2/Keap1 pathway by oral Dimethylfumarate administration alleviates oxidative stress and age-associated infertility might be delayed in the mouse ovary. Reprod Biol Endocrinol. 2019;17(1):23.
176. Niringiyumukiza JD, Cai H, Chen L, et al. Protective properties of glycogen synthase kinase-3 inhibition against doxorubicin-induced oxidative damage to mouse ovarian reserve. Biomed Pharmacother. 2019;116:108963.
177. Soylu Karapinar O, Pinar N, Ozcan O, et al. Protective effect of alpha-lipoic acid in methotrexate-induced ovarian oxidative injury and decreased ovarian reserve in rats. Gynecol Endocrinol. 2017;33(8):653–9.
178. Zhang J, Fang L, Shi L, et al. Protective effects and mechanisms investigation of Kuntai capsule on the ovarian function of a novel model with accelerated aging ovaries. J Ethnopharmacol. 2017;195:173–81.
179. Ozcan P, Ficicioglu C, Kizilkale O, et al. Can coenzyme Q10 supplementation protect the ovarian reserve against oxidative damage? J Assist Reprod Genet. 2016;33(9):1223–30.
180. Barbieri RL, Gochberg J, Ryan KJ. Nicotine, cotinine, and anabasine inhibit aromatase in human trophoblast in vitro. J Clin Invest. 1986;77(6):1727–33.
181. Elsherbiny ME, Brocks DR. The ability of polycyclic aromatic hydrocarbons to alter physiological factors underlying drug disposition. Drug Metab Rev. 2011;43(4):457–75.
182. Michnovicz JJ, Hershcopf RJ, Naganuma H, et al. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N Engl J Med. 1986;315(21):1305–9.
183. Bancroft J, Cawood EH. Androgens and the menopause; a study of 40-60-year-old women. Clin Endocrinol. 1996;45(5):577–87.
184. Li N, Fu S, Zhu F, et al. Alcohol intake induces diminished ovarian reserve in childbearing age women. J Obstet Gynaecol Res. 2013;39(2):516–21.
185. Schliep KC, Zarek SM, Schisterman EF, et al. Alcohol intake, reproductive hormones, and menstrual cycle function: a prospective cohort study. Am J Clin Nutr. 2015;102(4):933–42.
186. Schliep KC, Schisterman EF, Wactawski-Wende J, et al. Serum caffeine and paraxanthine concentrations and menstrual cycle function: correlations with beverage intakes and associations with race, reproductive hormones, and anovulation in the BioCycle Study. Am J Clin Nutr. 2016;104(1):155–63.
187. Faubion SS, Sood R, Thielen JM, et al. Caffeine and menopausal symptoms: what is the association? Menopause. 2015;22(2):155–8.
188. London S, Willett W, Longcope C, et al. Alcohol and other dietary factors in relation to serum hormone concentrations in women at climacteric. Am J Clin Nutr. 1991;53(1):166–71.
189. Freeman EW, Gracia CR, Sammel MD, et al. Association of anti-mullerian hormone levels with obesity in late reproductive-age women. Fertil Steril. 2007;87(1):101–6.

190. Boutot ME, Purdue-Smithe A, Whitcomb BW, et al. Dietary protein intake and early menopause in the Nurses' Health Study II. Am J Epidemiol. 2018;187(2):270–7.
191. Kanwal R, Gupta K, Gupta S. Cancer epigenetics: an introduction. Methods Mol Biol (Clifton, NJ). 2015;1238:3–25.
192. Zhang XF, Zhang LJ, Li L, et al. Diethylhexyl phthalate exposure impairs follicular development and affects oocyte maturation in the mouse. Environ Mol Mutagen. 2013;54(5):354–61.
193. Li L, Zhang T, Qin XS, et al. Exposure to diethylhexyl phthalate (DEHP) results in a heritable modification of imprint genes DNA methylation in mouse oocytes. Mol Biol Rep. 2014;41 (3):1227–35.
194. Zhang XF, Zhang T, Han Z, et al. Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reprod Fertil Dev. 2015;27 (8):1213–21.
195. Patel BB, Raad M, Sebag IA, et al. Lifelong exposure to bisphenol a alters cardiac structure/function, protein expression, and DNA methylation in adult mice. Toxicol Sci. 2013;133 (1):174–85.
196. Zhang XF, Zhang LJ, Feng YN, et al. Bisphenol A exposure modifies DNA methylation of imprint genes in mouse fetal germ cells. Mol Biol Rep. 2012;39(9):8621–8.
197. Zama AM, Uzumcu M. Fetal and neonatal exposure to the endocrine disruptor methoxychlor causes epigenetic alterations in adult ovarian genes. Endocrinology. 2009;150(10):4681–91.
198. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen. 2017;58(5):235–63.
199. Simon AM, Goodenough DA, Li E, et al. Female infertility in mice lacking connexin 37. Nature. 1997;385(6616):525–9.
200. Ackert CL, Gittens JE, O'Brien MJ, et al. Intercellular communication via connexin43 gap junctions is required for ovarian folliculogenesis in the mouse. Dev Biol. 2001;233(2):258–70.
201. Paksy K, Rajczy K, Forgacs Z, et al. Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. J Appl Toxicol. 1997;17(5):321–7.
202. Sharovskaya J, Kobliakova I, Solomatina N, et al. Effect of some carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons on gap junction intercellular communication in hepatoma cell cultures. Eur J Cell Biol. 2006;85(5):387–97.
203. Fiorini C, Tilloy-Ellul A, Chevalier S, et al. Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants. Reprod Toxicol (Elmsford, NY). 2004;18 (3):413–21.

Chapter 5
Effects of Environment and Lifestyle Factors on Anovulatory Disorder

Ying Song and Rong Li

Abstract Anovulatory disorder comprises around 30% of female infertility. The origin of ovulatory failure is rooted in pituitary FSH secretion. Any factor or process that disrupts the finely tuned interactions of hypothalamo–pituitary–ovarian axis can potentially lead to anovulation. The World Health Organization (WHO) has classified anovulatory disorders into three categories: hypothalamic–pituitary failure, hypothalamic–pituitary dysregulation, and ovarian failure. Due to industrial development, environmental pollution, and global warming, the human living environment has undergone tremendous changes. Industrial waste, noise, pesticides, fertilizers, and vehicular emission are visible pollutants responsible for environmental contamination and ill effects on health of all living systems. A considerable body of research suggests that chemical exposures in the environment or workplace may be associated with endocrine disruption of the synthesis, secretion, transport, binding, or elimination of natural hormones. For instance, some advanced biological mechanisms suggest that heavy metals may affect progesterone production, which possibly disturbs endocrine function in pregnant women. On the other hand, our lifestyle factors have also changed accordingly, which greatly influence overall health and well-being, including fertility. Many lifestyle factors such as nutrition, weight, exercise, and psychological stress can have substantial effects on female ovulation.

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5.1 Introduction

Ovulation is a process in which the oocyte matures and exits from the follicle. This precise process requires cooperation among the hypothalamus, pituitary gland, and ovary. Other endocrine organs, such as adrenal gland, thyroid gland, and even adipose tissue, may also have an impact on this process. Any factor or process that disrupts finely tuned interactions of hypothalamo–pituitary–ovarian axis can potentially lead to anovulation. Anovulatory disorder comprises around 30% of female infertility. Over the past few decades, human fertility has shown a significant decline trend, and environmental degradation may be one of the reasons. A great deal of evidence has been gathered to prove that certain chemical, physical, and biological substances present in our environment have harmful effects on human reproduction. At the same time, modern lifestyles are very different from before. However, the harmful effects of bad lifestyles on the female reproductive system cannot be underestimated, ranging from hormonal imbalances, low ovarian function, prolonged conception, to premature ovarian failure, ovulation disorders, and even infertility. Current research has found that the effects of obesity, underweight, and eating disorders, smoking, excessive exercise, drinking, caffeine intake, drug use, and even social work stress on the female reproductive system are particularly significant. Environment and lifestyle factors play key roles in determining reproductive health. Anovulatory disorder is one of the main manifestations.

5.2 Ovulation

Ovulation is a process in which the oocyte matures and exits from the follicle. Normally, the human ovary produces a single dominant follicle that ovulates in each menstrual cycle, which begins with the onset of puberty and diminishes when the follicular pool becomes depleted. After menopause, the ovaries generally no longer ovulate, and become smaller with the connective tissues proliferate. After a woman is born, there are about 300,000 to 600,000 follicles on both sides of the ovary, but only approximately 300–400 oocytes will be ovulated during a woman's reproductive lifetime and the rest will degenerate [1]. Maintenance of the primordial follicle pool, follicle recruitment and selection, dominant follicles development, oocyte maturation, atresia of other follicles is a quite complex process, which is determined by complex activation and interplay of many factors acting in a stage-specific manner. This involves a large number of compounds, endocrine, autocrine, and paracrine factors including inhibin, follistatin, activin, insulin like growth factor (IGF), IGF binding proteins, transforming growth factor family (TGF), epidermal

growth factor, endothelins, etc. This precise process requires cooperation among the hypothalamus, pituitary gland, and ovary. Other endocrine organs, such as adrenal gland, thyroid gland, and even adipose tissue, may also have impacts on this process [2].

5.2.1 Folliculogenesis

Follicle development begins with the collection of primitive follicles into a growing follicle pool. After a series of selection and growth, it ends with ovulation or apoptotic arrest, a process that can take up to a year in human. The interaction between the oocyte and the somatic cell is the most critical process. Two distinct stages could be divided from primordial to the preovulatory stage for follicle growth based on its responsiveness to the gonadotropins:

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The first stage is from primordial follicle to primary preantral follicle, which has been considered to be gonadotropin-independent and essentially controlled by locally produced growth factors operating through autocrine/paracrine mechanisms. Some growth mediators may play a role at this early stage, such as TGF-β, bone morphogenetic proteins (BMPs), activin, insulin, anti-Müllerian hormone (AMH), estrogen, and androgens [3, 4].

The second stage is the progression throughout the antral stage and ovulation, which have been considered to be dependent on pituitary-secreted gonadotropin (FSH and LH) support. Insulin growth factor (IGF) family, TGF-β family, growth differentiation factor-9 (GDF9), and BMP15 also play critical roles in these stages [4]. Follicles containing fully-grown oocytes are ready to undergo ovulation, which is induced by the surge of gonadotropins, which activates a whole cascade of inflammatory responses in the dominant follicle leading to the rupture of the follicular boundary wall and the release of the cumulus–oocyte complex. Besides steroids, GDF9, BMP15, and other proteins such as FOXL2 and NOBOX also seem to be involved in this process [5]. In addition, apoptosis plays an important role in the determination of the original follicle pool and the selection of dominant follicles. Many pro-apoptotic and anti-apoptotic proteins have been shown to regulate the growth and development of germ cells [6].

5.2.2 Oogenesis

Oogenesis is a process in which primary oocytes that are stagnant during the first prophase of diplotene stage of embryonic life reach meiotic maturity and undergo cytoplasmic changes, eventually in the form of mature eggs in reproductive life freed. The migration of the germ cells from the yolk sac to the genital ridge leads to oogenesis, which starts in the sixth week of embryo. Since reaching the genital ridge,

primordial germ cells proliferate and differentiate to oogonia. With GREL cells proliferation and surrounding the oogonia, primordial follicles arise, composed of one oocyte surrounded by a layer of flattened granulosa cells. And then, the granular cells become cuboids and multiply into multiple layers, and the follicles also develop from primary follicles to secondary follicles. The first primary follicles appeared in 15th to 16th weeks, while the earliest sinus follicles were observed in 23rd to 24th weeks. By the eighth month of the fetus in the womb, almost all germ cells become primary oocytes, which have entered meiosis and stagnated in the diplotene stage. Stopping mitosis of oocytes indicates that the number of oocytes reaches the peak in the whole life. In childhood, the ovaries are almost stationary. After puberty, reactivation of the GnRH pulse results in increased gonadotropin release and ovarian stimulation [7].

Oocytes remain at the dictyate stage of meiosis I throughout oogenesis, until final oocyte maturation, but it is not static. During this period, oocytes synthesize and accumulate RNAs and proteins that are essential for their proper growth and maturity, which are also essential for developing a viable embryo. The size and volume of oocytes has increased approximately 100-folds. From primitive to primary follicular phase, most genes related to cell proliferation, cell cycle, and transcription are all up-regulated. From the primary follicular phase to the secondary follicular phase, many transcripts that are involved in the cell cycle, biosynthesis, and macromolecular metabolism are actively up-regulated [8]. And then until to the sinus phase, genes involved in the basic transcription of polymerase II promoter are down-regulated, which may explain massive transcriptional silencing at the end of oocyte growth. The surge of FSH and LH before ovulations promotes the recovery of primary oocytes and completes the first meiotic division, forming secondary oocytes and polar bodies. The secondary oocyte enters the second meiosis and remains in the middle of meiosis until fertilization. Fertilization leads to the final completion of meiosis and the formation of a second polar body [9]. However, not all primary oocytes reach the mature egg stage, and most oocytes become atretic during this process [10, 11].

Oogenesis is a complex process, which is regulated by a vast number of intra- and extra-ovarian factors. Communication between the oocyte and the surrounding granulocytes is critical for oocyte development. Differentiated granulocytes provide oocytes with the nutritional and regulatory signals needed to promote oocyte nuclear and cytoplasmic maturation and thus acquire developmental capacity [11, 12]. The meiotic stage is essential for oocyte development, ovulation, and the formation of healthy embryos. In fact, it has been shown that endocrine-disrupting chemicals, such as bisphenol A (BPA), can cause spindle formation disorders, interfere with microtubule polymerization, and induce multipolar spindles in mouse oocytes [13].

5.3 Etiology of Anovulation Disorder

The release of a mature and fertilizable oocyte from a dominant follicle is the perfect integration of hypothalamic, pituitary, and ovarian functions. The main players of this system are GnRH, FSH, LH, estrogen, and progesterone, but countless other factors also provide the necessary regulation. In general, the normal function of the hypothalamic–pituitary–ovarian axis depends on the release time and the correct synchronization of the amount of hormones involved, which is essential for ovulation. Any factors or processes that interfere with the fine-tuning interaction of the hypothalamic–pituitary–ovarian axis may lead to anovulation.

5.3.1 Hypothalamic Factors

Hypothalamic hormones particularly gonadotropin-releasing hormone (GnRH) is an important factor responsible for functional hypothalamo–pituitary–ovarian axis. Fluctuations in the frequency and amplitude of GnRH pulsation release are the key to determine the FSH and LH release patterns, which in turn determine the triggering of ovulation. Studies have shown that the ability of GnRH to up-regulate the pituitary gonadotropin receptor requires a 60–90 min physiological cycle, and slower or faster frequencies can lead to anovulation [14]. GnRH hormone is a decapeptide synthesized and released from the specialized neuron ends of the hypothalamic arcuate nucleus. Its secretion is regulated by the positive and negative feedback of the pituitary and ovaries, as well as by autocrine and paracrine. GnRH secretion by the hypothalamus is regulated by dopamine, norepinephrine, serotonin, and opioids produced in the brain. Norepinephrine stimulates GnRH release, while dopamine and opioids inhibit GnRH release. Kisspeptins also affect the regulation of GnRH secretion [15, 16].

Hypothalamic dysfunction and structural abnormalities may affect GnRH production and release, thus leading to anovulation. Functional hypothalamic anovulation (FHA) is the most common cause of hypothalamic anovulation. Vigorous exercise, excessive stress, anxiety, malnutrition, and eating disorders can inhibit normal GnRH pulsation by inhibiting the excessive release of corticotropin-releasing hormone, and stimulation of β-endorphin can cause amenorrhea and anovulation [17]. Sedatives, antidepressants, stimulants, and antipsychotics can alter the levels of norepinephrine, dopamine, and serotonin, which in turn affect the release of GnRH leading to anovulation. Drug abuse (e.g., cocaine, marijuana) and mental illness (e.g., schizophrenia) can also cause anovulation by inhibiting GnRH [18]. Besides, infiltrative disorders of the hypothalamus (e.g., lymphoma, sarcoidosis), infection, head trauma, tumors of hypothalamus, irradiation to the hypothalamus, chemotoxic agents interfere with local neurotransmitter regulation of GnRH pulsatility can cause ovulation disorder.

5.3.2 Pituitary Factors

As we have known, pituitary hormones, especially Gn, play key roles in follicle formation and ovulation. GnRH in the hypothalamus is transported through the portal circulation to the anterior pituitary gland, causing the release of gonadotropins (LH and FSH). Gonadotropin-releasing cells account for 7–15% of the anterior pituitary cells. The ovaries release estradiol, progesterone, and inhibin, while the pituitary releases activin and follistatin, all of which regulate LH and FSH secretion during the menstrual cycle.

Hypophysectomy and radiotherapy are the commonest pituitary causes for pituitary tumors and Sheehan's syndrome (severe post-partum hemorrhage). Pituitary infiltrates (sarcoidosis, hemochromatosis), space occupying lesions (microadenomas, large adenomas), brain tumors (meningiomas, gliomas, and cranial neuromas), damage and radiation lead to pituitary destruction and anovulation. Not a few cases are idiopathic [19].

5.3.3 Ovarian Factors

The ovaries are responsible for the production and periodic release of oocytes and the production of estradiol and progesterone. Radiotherapy, chemotherapy, and ovariectomy are iatrogenic factors that directly damage the ovaries, leading to anovulation and infertility. Chromosomal abnormalities, such as Turner's syndrome, fragile X syndrome, idiopathic accelerated ovarian follicular atresia, and gonadal hypoplasia, are the genetic causes of anovulation. Premature ovarian failure and ovarian resistance syndrome are other causes of anovulation, and their causes are mostly unknown [20].

5.3.4 Endocrine Factors

Hyperprolactinemia caused by pituitary tumors or drugs can cause anovulation by affecting multiple parts of the hypothalamic–pituitary axis. It mainly interferes with two factors: pulsed release of GnRH, positive feedback effect of estrogen on LH surge. Causes of hyperandrogenemia, such like Cushing's syndrome, adrenal hyperplasia, drug-induced virilization, and androgen-secreting tumors can lead to anovulatory. Severe thyroid dysfunction, both hyper- or hypo-thyroidism, if untreated, can cause anovulatory and menstrual irregularities.

5.3.5 Genetic and Epigenetic Factors

Genetic diseases, such as Kalman Syndrome, Prader–Willi Syndrome, and GnRH receptor gene mutations, can affect the production, release, and function of GnRH, leading to anovulation. Idiopathic gonadotropin deficiency hypergonadism, isolated gonadotropin deficiency, and mutations in the genes of FSH and LHβ subunits cause ovulation and infertility by affecting FSH and LH. Chromosomal abnormalities, such as Turner syndrome, Fragile X syndrome, idiopathic ovarian follicular atresia, and hypogonadism are genetic factors that cause ovulation to lack ovaries [21].

Epigenetic mechanisms participate in the regulation of gene expression in the condition of not changing DNA sequence. Environmental factors are involved in these mechanisms and lead to chromatin structural changes. Methylation and demethylation, histone acetylation, ATP dependent chromatin remodeling, and DNA methylation are the most typical mechanisms in epigenetic regulation. In reptiles, temperature is an environmental stimulus that affects sex determination by changing the DNA methylation pattern in the aromatase gene promoter, which seems to be essential in the commitment to the female pathway [22]. On the other hand, environmental toxicants, such as PCBs, may cause epigenetic changes, leading to changes in the expression profile of genes related to gonad differentiation in turtles [23]. Recently, the impact of epigenetics on human ovulation has become a research hotspot. The best epigenetic reprogramming cycle is the cycle of DNA methylation. The life cycle of this epigenetic marker includes several key stages: clearing of epigenetic markers from primitive germ cells; the establishment of a new set of markers during gamete development; elimination of genome-wide methylation at the pre-implantation stage. Starting from the blastocyst stage, markers are re-established during development and differentiation [24]. Advanced age, body composition, diet, genetic/epigenetic variation, and environmental exposures have been proved to have effect on epigenetic programming of the mammalian germline.

5.3.6 WHO Classification

According to the main causes of anovulation, and based on the levels of gonadotropins and estrogen in the peripheral blood, the World Health Organization (WHO) has developed a classification standard for anovulation diseases. Anovulatory diseases are divided into three categories, namely hypothalamic–pituitary failure, hypothalamic–pituitary dysfunction, and ovarian failure. [25].

WHO Group I (Hypothalamus–Pituitary Failure). This situation may be caused by hypothalamic or pituitary disease, or severe weight loss or excessive stress or high-intensity exercise. The typical performance is hypogonadotropic hypogonadism: the serum gonadotropin (LH and FSH) concentration is too low, the development of follicles in the ovary is blocked, which in turn leads to anovulation and amenorrhea, the level of estrogen is significantly reduced.

WHO Group II (Hypothalamic–Pituitary Dysfunction). This is the most common type of anovulation, accounting for about 90% of all anovulatory patients, most of which are caused by polycystic ovary syndrome (PCOS). Also includes follicular membrane cell hyperplasia and HAIRAN syndrome. In this type of anovulation, women's serum gonadotropin and estradiol levels are normal, but LH/FSH is often abnormally elevated. That is to say, the ovarian function disorder with normal gonadotropin is accompanied by anovulation or oligomenorrhea in varying degrees. The occurrence and growth of follicles can be observed, but the follicles cannot mature and cannot spontaneously ovulate.

WHO Group III (Ovarian Failure). This condition is mainly due to severe lack of primordial follicles or ovarian resistance, including primary or secondary. Manifestations of hypogonadotropic hypogonadism: increased serum FSH and LH, and decreased E2. The clinical manifestations of the patients are amenorrhea, infertility, and low estrogen-induced perimenopausal symptoms. This type of patient is characterized by a poor response to induced ovulation, and ovarian function has deteriorated.

5.4 The Influence of Environmental Factors on Female Ovulation

Many evidence show that environmental factors may play substantial roles in the development or cause of disease. A cohort study of nearly 45,000 twins from Denmark, Sweden, and Finland showed that compared to genetic factors, environmental factors may play more important role in the onset of cancers such as breast, prostate, and others of female reproductive system [26]. There are now more than 87,000 known chemical substances in the United States, penetrating our food, air, soil, water, homes, schools, transportation systems, and workplaces [27]. Our human fertility experienced obvious decline over the past decades, and environmental degradation may play a role in the procedure. Many evidence has proven that certain physical, chemical, and biological substances in the environment may be harmful to human reproduction. Female fertility may be affected by environmental factors in coexisting ways, such as epigenetic modification, induction of oxidative stress, and endocrine disruptors.

5.4.1 Climate Change

Since the industrial period, human activities have led to significant emissions of carbon dioxide and other greenhouse gases, which have generated rapid variations in atmospheric composition and driven major climate changes. Different aspects of global climate change, such as the rise in ambient temperature over the past 30 years,

have shown a wide range of biological systems. An important aspect of biosystem affected by climate change is phenology. More and more studies show that the reproductive function of animals has been greatly influenced by climate change, especially fish and birds. However, there are few studies on climatic factors and human reproductive functions, especially ovulation, which is related to excessive external interference factors [28, 29].

Climate change includes the change of air composition, sunshine cycle, and the most prominent is the rise of temperature. Climate change will continue to have an increasingly dramatic effect on the global thermal environment, including increases in average local temperatures and the frequency of heatwaves [30]. Increase of environmental temperature will cause increase of body temperature, and thus increasing metabolic heat production. Hyperthermia could also trigger lethargy, possibly through triggering central fatigue involving dopamine and serotonin level changes, resulting in decreased activity and thus reduced heat production. Long-term exposure to high temperatures could reduce basal heat production of mammals by reducing circulating thyroid hormone, which is a common response to various environmental stresses. Generally, the endocrine responses to heat stimulation include the plasma concentration of several hormones such as adrenaline, glucocorticoids, noradrenaline, and brain oxytocin. HPO axis could also be affected by these hormonal changes, leading to anovulatory disorders. Because of its sensitivity to energy availability, reproductive axis of mammals could be directly affected by high temperatures and heat waves [31, 32].

Research has shown that high temperature can reduce the intermittent secretion of LH, inhibit the surge of LH before ovulation, and thus reduce the gonadotropin dependent growth of follicles [33].

Research confirms that photoperiod is an important factor in coordinating reproductive and environmental synchronization. Melatonin, which depends on photoperiod, is central to the ovulatory rhythmic endocrine regulation system. Melatonin is an endocrine regulator with a wide range of functions secreted by the pineal gland. It regulates female reproductive endocrine and ovulation through the effect on the H-P-O axis [34].

5.4.2 Ionizing Radiation

Ionizing radiation can target rapidly dividing cells in multiple organs, including the testes and ovaries, as well as the developing embryo and fetus. The effects of radiation on the human reproductive system have been extensively explored through events of large population exposure. The accident at Chernobyl nuclear power station on April 26, 1986 was the most serious accidental radiation leakage in the twentieth century. A series of cases of Down's syndrome in Belarus 9 months after the explosion of the Chernobyl nuclear power station indicate that mammals are in a radiosensitive period of oogenesis during ovulation and conception [35]. Ionizing radiation would lead to production of free radicals, therefore increase oxidative

stress in cells or tissues. Cell macromolecules, even nuclear DNA could be damaged by those free radicals. Most of single or double strand breaks of DNA damage could be repaired in a few hours. Compared to double chain breaks, single chain breaks which are usually caused by low LET radiation, are easier to be repaired. Also, double strand breaks have harmful effect on genome integrity [36–38]. Double strand breaks of DNA by irradiation also have been shown to play important role in triggering the mitochondrial apoptotic pathway [39]. The effect of oxidative stress state on cells not only happened during radiation exposure, but also long time after exposure.

Radiotherapy for cancer patients will lead to radiation-induced harm, which may have a profound impact on female reproductive function. Ovarian toxicity is a common long-term side effect of therapeutic radiotherapy. Anovulation of the ovary after radiation is caused by the inactivation of oocytes and the decrease in the number of granulocytes, which causes oocyte maturation microenvironment abnormalities [40]. Previous studies have shown a significant correlation between follicular developmental stage and oocyte damage after irradiation. The effect of radiation on female gamete and ovulation rate is not only affected by the development stage, but also time-dependent. During fetal period, radiation is easy to cause serious effects on the mitotic oocytes. Now more and more data show that in the primordial, primary, and antral stages, the number of follicles will be significantly reduced after receiving radiation, and the follicles in preantral stage are better tolerated by radiation [41].

To assess the effects of radiation on the ovarian function of women, the age of the patient, the time of exposure, and the total dose should be taken into account. Generally speaking, due to the large number of follicles in the ovaries before puberty, the follicles in the ovaries are less prone to apoptosis than middle-aged women. However, because the radiation source is too close to the ovary, even young women undergoing abdominal radiotherapy may cause a significant decrease in ovarian function. In terms of the total radiation dose, it is estimated that the harm of less than 60 cGy to the ovary can be ignored. When the total dose reaches 150 cGy, there is a risk of decreased ovarian function for women over 40 years of age, but not for young women. At a dose of 250–500 cGy, 60% of 15–40-year-old women experience permanent sterility, the rest may experience temporary amenorrhea and anovulation, and while women over 40 years old may experience 100% permanent sterilization [42–44].

Another potential reason of anovulation caused by radiation damage to the reproductive system is the destruction of the H-P-O axis. In therapeutic cranial irradiation, this damage can occur if the hypothalamus and pituitary are within the scope of radiation therapy. The function of H-P-O axis may change in different degrees in patients with brain tumor undergoing local radiotherapy. In the case of severe injury, gonadotropin and estrogen in the circulation of the patients decreased significantly, and the clinical manifestations were anovulation. Some case reports show that precocious puberty occurs in children who have received radiotherapy for leukemia or brain tumors [45]. Precocious puberty after radiotherapy may be due to the damage of inhibitory feedback system in H-P-O axis. Thus, the release of GnRH

in hypothalamic neurons is activated prematurely, and then the amplitude and frequency of GnRH pulsates are both increased.

Electromagnetic radiation is widely available both indoors and outdoors, such as satellite links, wireless communications, microwave ovens, FM radios, and TV transmitters/antennas, etc. Wi-Fi signals are ubiquitous in the home or office, used for wireless applications and Internet connection, thereby increasing exposure to the radiations [46]. Studies show that exposure to Wi-Fi signals can increase the generation of reactive oxygen species [47] and adversely affect oocyte development and ovulation [48]. There is another problem. Mobile phones are revolutionary to human life and are indispensable. In mobile phone communications and many other applications, exposure to electromagnetic radiation is increasing. The electromagnetic radiation produced by mobile phones is non-ionizing radiation, but the effects on the reproductive system cannot be completely denied.

5.4.3 Environmental Endocrine Disruptors

One of the greatest achievements of the twentieth century was the ability to replicate and synthesize organic compounds, from synthetic rubber to drugs of all kinds. These substances play a very important role in promoting the development of human civilization, but people gradually realize that they bring some potential hazards. The problem of endocrine interferon is gradually recognized. Endocrine-disrupting chemicals (EDCs) are exogenous chemical entities or mixtures of compounds that interfere with any aspect of hormone action responsible for the maintenance of homeostasis and the regulation of developmental processes [49]. In the past two decades, the research on potential harmful effects of EDC on human body has been greatly increased, which makes the knowledge of developing biological blood and environmental toxicology continuously increases. The toxic effects of EDCs have led to restrictions on their use with substantial evidence of exposure. Some western countries prohibited the use of some of the EDCs, such as polybrominated diphenyl or polychlorinated biphenyls. However, sometimes human exposure to EDCs is inevitable, for example, when those chemicals are used in the occupation, or, when those chemicals are widely dispersed in the environment [50, 51].

EDC changes the interaction between gene and environment through physiological, cellular, molecular, and epigenetic changes. EDCs have an impact on the exposed individuals and their offspring. The biological effect mechanism of EDCs is mainly to combine the corresponding endogenous hormone receptor with hormone competition, so as to promote/antagonize hormone action, leading to body dysfunction; to interfere with the synthesis and metabolism and transport of endogenous hormone and its receptor, leading to endocrine dysfunction. They can cause multiple damages to women's hypothalamus, pituitary, breast, uterus, ovary, and endocrine system and induce a variety of adverse outcomes and complications. This damage spans almost every stage of female life, from the embryonic development stage to Adolescence, childbearing age, and menopause [52, 53]. Although there

may be hundreds or more environmental chemicals with EDC activity, several categories are most frequently studied and will be briefly introduced here [49, 54].

5.4.3.1 Bisphenol A

Bisphenol A (BPA) was first synthesized in 1891 and found to have estrogen activity in 1936. It is widely used in manufacturing, food packaging, toys, and other applications, becoming the largest annual production of EDCs. BPA is contained in the lining of many canned foods and beverages, which will seep into food or water under high temperature, physical operation, or repeated use, causing everyone to be exposed. Although the half-life of BPA is relatively short (6–24 h), it can be measured in reproductive tissues such as follicular fluid, breast milk, placenta, etc., [55].

It has been proved in animal models that BPA could affect the growth and development of follicles and ovulation. Although the effects of BPA on follicular dynamics and/or oocyte maturation are two closely related physiological processes, they may vary according to the period and mode of exposure, specie, period of observation. They are frequently reported in different animal species, including sheep [55]. Low doses of BPA exposure in the uterus is interfered with early oogenesis production in rhesus monkey and mice, and, injection of BPA induces MOFs after birth. MOFs are follicles which contain two or more oocytes without a separating basement membrane. The formation of MOF during nest breakdown seems to be due to incomplete breakdown, which could be used as an indicator for the obstruction of primordial follicle formation [56, 57]. In addition, it can reduce the number of primordial follicles, increase the number of apoptotic oocytes, and promote the recruitment of primordial follicles. At the same time, reducing the number of antral follicles and increasing the number of primary and secondary follicles affected the distribution of follicle type [58, 59]. In many experimental studies of rats and mice in different life stages, BPA has been found to change the estrous cycle. Proper periodicity is considered essential for successful ovulation. Therefore, periodic changes may directly lead to at least sub-fertility through disturbed ovulation (delay or absence) [60].

Among infertile women undergoing IVF, higher BPA exposure (higher BPA in blood or urine) is associated with lower ovarian response, reduced number of mature oocytes, and decreased number of fertilized oocytes. Thus, increased levels of BPA may decrease the success rate of IVF treatments [55, 61–63]. In addition, BPA exposure is also closely related to polycystic ovary syndrome (PCOS). PCOS is one of the most common diseases in reproductive women, which is caused by the abnormal endocrine and metabolism in the body. It is mainly manifested as irregular menstrual cycle, infertility, acne, hirsutism, accompanied by abnormal gonadotropin-releasing hormone secretion and high androgen level, affecting the normal development of follicles, causing ovulation disorder and difficult to conceive. A large number of studies have shown that exposure to EDCs can lead to

impaired physiological functions of the ovary and affect the occurrence and development of PCOS disease [64, 65].

Molecular analysis showed that low dose of BPA could affect the level of apoptosis related genes. Specifically, BPA can increase the level of B-cell leukemia/lymphoma 2 (Bcl2), BCL2-like 1 (Bcl2l1), decrease the level of BCL2 antagonist/killer 1 (Bak1), tumor necrosis factor (TNF) receptor superfamily and lymphotoxin B receptor (Ltbr) [66, 67]. In addition, BPA exposure reduced the expression of factors that control follicular formation, such as LIM homeobox protein 8 (Lhx8), NOBOX oogenesis homeobox(Nobox), spermatogenesis and oogenesis-specific basic helix-loop-helix 2 (Sohlh2), stimulated by retinoic acid gene 8 (Stra8), REC8 meiotic recombination protein (Rec8), synaptonemal complex protein 3 (Scp3), DNA meiotic recombinase 1 (Dmc1), and folliculogenesis-specific basic helix-loop-helix (Figla) [68, 69]. In addition, BPA exposure prevented DNA methylation at the Lhx8 CpG site [69]. All these evidences suggest that BPA may impair normal follicular formation and ovarian dynamics.

5.4.3.2 Phthalates

Phthalates are a large class of compounds, which are widely used in plastics, coatings, cosmetics, medical pipes and other products. They are produced in large quantities because they give the material a certain degree of flexibility and flexibility. The most common one is di(2-ethylhexyl) phthalate (DEHP), whose active metabolite is mono (2-ethylhex-yl) phthalate (MEHP). In the 1920s, these compounds were first introduced into plastics production as additives. Through oral, inhalation, or skin contact, human exposure to phthalates is ubiquitous. Phthalates have been described in many studies as endocrine disruptors that alter ovarian function, by influencing folliculogenesis and steroid production. When considering fetal development, exposure of pregnant mice to MEHP will cause premature ovarian failure in F1 generation [70]. According to the acceleration mechanism of follicle recruitment, it leads to the exhaustion of the primordial follicle pools of F1 and F2 generations. This multigenerational effect could be explained by the effect of phthalates on DNA methylation of imprinted genes, not only in fetal ovarian germ cells, but also in F1 and F2 offspring [71]. In one study, the number of preantral follicles in offspring increased on the 21st day after birth.

The authors explain that this growth is due to the accelerated growth of primordial follicles and primary follicles, leading to premature failure of ovarian function [72]. Similarly, exposure to phthalates before puberty significantly reduces the number of primordial follicles during puberty and adulthood by accelerating follicular recruitment [73, 74]. Mechanism study showed that when exposed to DEHP, the content of apoptosis promoting gene messenger RNA increased, which caused oxidative stress and apoptosis of ovarian somatic cells [75].

5.4.3.3 Atrazine

Atrazine (2-chloro-4-ethylamino-6-isopropylamino)-s-triazine (ATR) is a common herbicide used to control the growth of broadleaf and grass weed on crops such as commercial corn, sorghum, and sugarcane. ATR was banned in 2004 in EU countries, however, it is still used for agricultural purposes in the United States, China, and Africa [76]. Humans are primarily exposed to ATR through contaminated farmland, surface water, and groundwater. Epidemiological studies show that women living in rural areas where ATR is widely used have irregular menstrual cycles, longer follicular periods, and lower levels of estradiol and progesterone metabolites [77].

ATR's reproductive toxicity and anti-ovulation effects have been demonstrated throughout animal studies. It has been reported that oral administration of high-dose ATR to adult female rats can reduce the LH surge and the length of estrus cycle, as well as the number of luteal and oocyte released. In FSH-stimulated rat granulosa cells, ATR reduced estradiol levels and aromatase (Cyp19a1) expression, also can selectively reduce LH receptor mRNA levels, resulting in low ovulation gene expression under hCG stimulation [78]. In addition, ATR induces overexpression of luteal markers, such as steroids producing acute regulatory protein (Star) and cytochrome P450 side chain lyase (Cyp11a1), followed by increased progesterone synthesis [79]. Similarly, studies have shown that targeting human cumulus granulosa cells, ATR can reduce the levels of estradiol and progesterone and prevent LH-dependent expression of ovulation genes [80].

5.5 Effect of Lifestyle Factors on Ovulation

Along with social and economic development, modern lifestyles are very different from before. However, the harmful effects of a bad lifestyle on the female reproductive system cannot be underestimated, ranging from hormonal imbalances, low ovarian function, prolonged conception, to premature ovarian failure, ovulation disorders, and even infertility [81]. Current research has found that the effects of obesity, underweight, and eating disorders, smoking, excessive exercise, drinking, caffeine intake, drug use, and even social work stress on the female reproductive system are particularly significant [82]. Ovulation disorder is one of the main manifestations. Lifestyle factors play important role in determining reproductive health and can have a positive or negative impact on fertility, but they are ultimately under our own control.

5.5.1 Diet

To maintain overall health, healthy and varied diet may play the key role. In which, some vitamins and food groups have greater impact on reproductive health than others. A woman's diet may ultimately affect her fertility, especially ovulation. About 12% of anovulatory infertility is caused by underweight. Underweight women often have anorexia nervosa, abnormal ovulation, and oligomenorrhea. The concentration of leptin in the circulation is very low in women who are underweight. This may be one of the factors that cause the reduction of GnRH secretion, which can cause the gonadotropin level to be too low to maintain the normal function of the ovaries [83].

The lifetime prevalence of anorexia nervosa in women is 0.9%, with the average age of onset being 19 years old. Anorexia nervosa is often associated with extreme restrictions on food intake and excessive exercise. Limiting calorie intake or increasing energy expenditure will result in a reduction in metabolic fuel. The levels of prolactin, estradiol, progesterone, testosterone, androgen, luteinizing hormone, and gonadotropin in the blood of women with anorexia nervosa decreased, while FSH levels increased significantly. Abnormal levels of these sex hormones cause menstrual disorders and ovulation dysfunction [83].

Not only should the diet be adequate, it should also be healthy and varied. Multivitamins help women ovulate, and those who take multivitamins have less possibility to have anovulatory infertility [84]. Studies show that animal protein is harmful to women's ovulation compared to carbohydrates (OR 1.18). Eating only one portion of meat increases the chance of anovulatory infertility by 32%, especially chicken. The replacement of carbohydrates with vegetable protein showed protective effect (OR 0.5). Trans fatty acids in the diet significantly increase the risk of anovulation. Ingestion of trans fats instead of carbohydrates was associated with a 73% increased risk of ovulation disorders (RR 1.73) [85]. Chavarro et al. found that monounsaturated fatty acids, vegetables, reduced blood sugar load, iron and multivitamin intake can reduce the incidence of anovulatory disorder [86, 87].

5.5.2 Obesity

The epidemic of obesity has recently become a serious problem, which may be partly due to an energy-rich diet and lack of physical exercise. Studies have shown that about 25% of anovulatory disorders in the United States were caused by being overweight or obese. BMI is often used to assess the effect of weight on fertility. BMI > 27 kg/m^2 or BMI < 17 kg/m^2 is associated with an increase in anovulatory infertility. When BMI > 30 kg/m^2 or BMI < 17 kg/m^2, there will be abnormal secretion of gonadotropin-releasing hormone in hypothalamus, luteinizing hormone in hypophysis, estrogen in follicles, and even anovulation may occur [88]. It has been speculated that these negative effects may be related to the change of follicle

microenvironment, which is different between obese women and normal weight women. Some differences may include increased levels of insulin, lactate, triglyceride, and C reactive protein in follicular fluid, and sex hormone-binding globulin (SHBG) may also reduce [89]. Studies have shown that the mechanism of ovulation disorders caused by obesity is closely related to insulin resistance. Insulin resistance will stimulate the production of androgens in the ovary and promote the aromatization of androgens to estrogen in the periphery, eventually affecting follicular development [90]. Fat cells can also produce some metabolic signals, especially leptin, which can affect the secretion of GnRH by the hypothalamus, thereby stimulating the secretion of gonadotropins and affecting ovulation [91, 92]. Thankfully, the negative effects of obesity on female ovulation seem to be reversible. For obese women who do not ovulate, weight loss can help to rebuild ovulation or help them to respond to ovulation promotion [93]. Clark et al. found that after an average loss of 10.2 kg, 90% of obese anovulatory women began to ovulate, and 52 in 67 women achieved a pregnancy [94]. In obese women with PCOS, even a small weight loss (5–10%) can significantly improve fertility [95].

5.5.3 Exercise

Women's regular physical activity and exercise are beneficial to overall health and well-being, especially for obese women [96]. However, if the energy intake cannot make up for the increase in exercise energy consumption, it can lead to the disorder of the hypothalamic–pituitary–ovarian axis, leading to anovulation and many other systemic diseases. Excessive physical exercise is related to abnormal menstruation and ovulation, and even affects the development of follicles, which has a negative impact on fertility. Studies have shown that the frequency, intensity, and duration of exercise are related to the risk of ovulation disorders [97].

Challenges imposed on the physiological system during energy deficiency lead to the redistribution of energy to processes necessary for survival, such as thermoregulation, movement, and cell maintenance, but away from the processes that are less important for survival, such as reproduction and growth. From the "generator" of hypothalamic reproductive function to the end of follicular secretion of ovarian steroids, the production and secretion of reproductive hormones altered at various levels of the reproductive axis. Women athletes who take part in high-intensity sports often have irregular menstruation, while the long-term negative energy balance and low body fat content make the ovarian function extremely vulnerable. The level of LH, prolactin, and estradiol in the serum of the women who experienced strenuous exercise but had little menstruation was lower [98, 99].

5.5.4 Cigarette Smoking

More than 4000 chemicals are contained in cigarettes, such as hydrocarbons, alcohols, phenols, aldehydes, heavy metals, etc. Smoking causes many potential health complications in women. Compared with non-smoking women, the incidence of anovulation in smoking women is significantly higher [100]. Smoking is associated with early depletion of the ovarian oocyte pool, and some studies have suggested a link between smoking and early menopause. Cigarette smoking can cause the loss of high quality oocytes and even premature ovarian failure. Compared to non-smokers, menopause in women who smoke will occur 1–4 years earlier, and this dose-dependent effect suggests the consumption of ovarian follicles by smoking [101]. In animal experiments, cigarette exposure can induce the loss of follicles, reduce the weight of ovaries, and reduce the number of original follicles and growing follicles, which mechanism is not to induce apoptosis, but to activate autophagy pathway [102]. Benzopyrene is an important chemical component in cigarette. Sobinoff found that exposure of ovary to benzopyrene will increase the activation of primordial follicles and atresia of developing follicles, leading to premature ovarian failure and anovulation [103]. In addition, smoking can have a series of effects on disruptions in hormone levels. Polycyclic aromatic hydrocarbons (PAHs) affect ovulation by acting on the ovary through the aryl hydrocarbon receptor (AHR) on the surface of granulosa cells. This receptor belongs to the family of transcription factors and activates the expression of Bax gene (an apoptosis promoting gene) and cytochrome P450, which transforms PAHs into more toxic molecules [102]. Compared with non-smoking women, the level of basic FSH in the blood of active smoking women increased by 66%, while that of passive smoking women increased by 39%. Smoking can also reduce the level of AMH, which indicates that the ovarian reserve function is decreased. The potential mechanism of smoking affecting follicular development and resulting in low fertility may be related to oxidative stress [104].

5.5.5 Alcoholism

Compared with non-drinking women, pregnancy rate decreased significantly with the increase of women's drinking times. A case–control study of 1050 women in the United States and Canada and 3833 control women who were diagnosed with infertility found that ovulation-related infertility was associated with alcohol intake [105]. The effect may be due to oxidative stress, hormone fluctuations, including the rise of estrogen level, which can reduce FSH, inhibit follicular development and ovulation, but many mechanisms are still unclear [106].

5.5.6 *Drug Use*

Marijuana is one of the most commonly used drugs in the world, and it works in both the center and the periphery, causing abnormal reproductive functions. Tetrahydrocannabinol, the main chemical component that causes marijuana's mental effects, can cause irregular ovulation and shortened menstrual cycle, thus interfering with female fertility [107]. The risk of anovulatory infertility in marijuana smoking women was significantly increased. In women, marijuana use can negatively affect hormonal regulation. In a short period of time, marijuana may cause a decrease in LH levels, but over a long period of time, hormone levels may remain unchanged due to the development of tolerance [108]. Another commonly used recreational drug is cocaine, which is a substance that can stimulate the peripheral and central nervous system, and can cause vasoconstriction and anesthesia [109]. Cocaine will also affect ovarian function and ovulation. Thyer et al. found that in non-human primates, cocaine can reduce the ovarian response to exogenous gonadotropins, which suggests that cocaine can have a direct effect on the ovaries. Directly and quickly interfere with ovulation of the ovary [110].

5.6 Psychological Stresses

Stress is an important part of any society, including physical, social, and psychological aspect. Although acute stress can cause transient neuroendocrine, metabolic, and behavioral responses to promote survival in the face of perceived challenges, chronic stress can cause allogeneic neuroendocrine and metabolic regulation and can also promote survival, but can damage acute and chronic health. Due to social pressure, testing, diagnosis, treatment, failure, unfulfilled desires, and even the financial costs associated with it, infertility is itself a pressure [111, 112]. Even if no fertility is required, anovulation and abnormal menstruation can increase psychological stress. Stress is the most common and often underestimated cause of reproductive dysfunction [113]. Stress-induced anovulation (SIA) is a typical type of functional hypothalamic amenorrhea, which can cause infertility and increase the burden of acute and chronic health problem. SIA/FHA is usually caused by mental stress and mild energy imbalances and represents an adaptive behavior, with changes in neuroendocrine patterns and metabolism promoting [114].

Sustained stress will lead to long-term activation of the limbic–hypothalamus–pituitary–adrenal (LHPA) axis, which leads to a series of neuroendocrine regulation, including inhibition of the hypothalamus–pituitary–gonad (HPG) axis and hypothalamic–pituitary–thyroidal (HPT) axis in women [111, 115]. Gonadal function directly depends on the pulsed secretion of GnRH from the hypothalamus. Changes in the frequency and amplitude of pulsatile GnRH secretion will affect the secretion of LH and FSH in the hypothalamus, which will adversely affect follicular growth and development. Therefore, GnRH inhibition is one of the common causes

of anovulation and amenorrhea. However, SIA/FHA is anovulation and amenorrhea caused by GnRH inhibition [116]. Women with SIA/FHA showed a series of neuroendocrine and metabolic changes, which reflected the integration of neuroanatomy and neurophysiology of the neural network of synchronous hypothalamic function. These changes in neurosecretion at least reflect the feedback sensitivity of hypothalamus to estradiol, cortisol, and thyroxine. The secretory patterns of growth hormone, prolactin, and melatonin also differed from those of normal women. Cortisol levels in FHA patients were significantly higher than in women during normal ovulation, and glucose levels also decreased, which reflected hepatic depletion of glycogen due to chronic limbic–hypothalamic–pituitary–adrenal (LHPA) axis activation [117, 118]. It can be seen that stress management, relaxation training, or psychological education may have long-term benefits to women's mental and physical health [14, 119].

5.7 Conclusions

Environmental and lifestyle factors play important roles in folliculogenesis and ovulation. Although there are more and more studies on the relationship between EDCs and female reproductive health, most of them are rodent like animals, and the experimental dose is far greater than the population exposure level. Such experimental results are difficult to be extrapolated to the population. So we need more epidemiological preschoolers and a large number of clinical data. To fully explore the influence of EDCs, we should investigate every stage from intrauterine exposure to puberty and childbearing age. With the deepening of understanding, people also realize that the exposure to EDCs is not a single substance, but a combination of multiple substances. At present, the impact of mixed exposure of EDCs on female ovulation is still a field to be explored. The influence of lifestyle factors on female ovulation is difficult to quantify, but it is easily regulated. The long-term impact on the female reproductive system needs to be further explored. It is also necessary to give guidance and suggestions on the protection of female fertility and on the reduction of the incidence of anovulation.

References

1. Oktem O, Urman B. Understanding follicle growth in vivo. Hum Reprod. 2010;25:2944–54.
2. Piprek RP. Molecular mechanisms of cell differentiation in gonad development. Cham: Springer; 2016.
3. Dewailly D, Robin G, Peigne M, et al. Interactions between androgens, FSH, anti-Müllerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. Hum Reprod Update. 2016;22(6):709–24.
4. Chou C-H, Chen M-J. The effect of steroid hormones on ovarian follicle development. Vitam Horm. 2018;107:155–75.

5. Rimon-Dahari N, Yerushalmi-Heinemann L, Alyagor L, et al. Ovarian folliculogenesis. In: Results & problems in cell differentiation, vol. 58. Cham: Springer; 2016. p. 167.
6. Yadav PK, Tiwari M, Gupta A, et al. Germ cell depletion from mammalian ovary: Possible involvement of apoptosis and autophagy. J Biomed Sci. 2018;25(1):36.
7. Sadler TW. Langman's medical embryology. 12th ed. Lippincott Williams & Wilkins, printed in China: Wolters Kluwer; 2011. p. 384.
8. Elkouby YM, Mullins MC. Coordination of cellular differentiation, polarity, mitosis and meiosis – New findings from early vertebrate oogenesis. Dev Biol. 2017;430(2):275–87.
9. Wear HM, Mcpike MJ, Watanabe KH. From primordial germ cells to primordial follicles: a review and visual representation of early ovarian development in mice. J Ovarian Res. 2016;9 (1):36.
10. Kawashima I, Kawamura K. Disorganization of the germ cell pool leads to primary ovarian insufficiency. Reproduction. 2017;153(6):R205–13.
11. Hsueh AJW, Kazuhiro K, Yuan C, et al. Intraovarian control of early Folliculogenesis. Endocr Rev. 2015;36(1):1–24.
12. Dumesic DA, Meldrum DR, Katz-Jaffe MG, et al. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. Fertil Steril. 2015;103(2):303–16.
13. Yang L, Baumann C, De La Fuente R. Mechanisms underlying disruption of oocyte spindle stability by bisphenol compounds. Reproduction. 2020;159(4):383–96.
14. Gordon CM, Ackerman KE, Berga SL, et al. Functional hypothalamic amenorrhea: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2017;102(5):1413–39.
15. Wahab F, Atika B, Ullah F, et al. Metabolic impact on the hypothalamic kisspeptin-kiss1r signaling pathway. Front Endocrinol. 2018;9:123.
16. Iwasa T, Matsuzaki T, Yano K, et al. The roles of kisspeptin and gonadotropin inhibitory hormone in stress-induced reproductive disorders. Endocr J. 2018;65(2):133–40.
17. Sowińska-Przepiera E, Andrysiak-Mamos E, Jarzbek-Bielecka G, et al. Functional hypothalamic amenorrhoea — diagnostic challenges, monitoring, and treatment. Endokrynol Pol. 2015;66(3):252–68.
18. Lania A, Gianotti L, Gagliardi I, et al. Functional hypothalamic and drug-induced amenorrhea: an overview. J Endocrinol Investig. 2019;42(9):1001–10.
19. Mikhael S, Punjala-Patel A, Gavrilova-Jordan L. Hypothalamic-pituitary-ovarian axis disorders impacting female fertility. Biomedicines. 2019;7(1):5.
20. Rudnicka E, Kruszewska J, Klicka K, et al. Premature ovarian insufficiency – aetiopathology, epidemiology, and diagnostic evaluation. Menopausal Rev. 2018;17(3):105–8.
21. Yatsenko SA, Aleksandar R. Genetics of human female infertility. Biol Reprod. 2019;3:3.
22. Martínez-Juárez A, Moreno-Mendoza N. Mechanisms related to sexual determination by temperature in reptiles. J Therm Biol. 2019;102400:85.
23. Mizoguchi BA, Valenzuela N. Ecotoxicological perspectives of sex determination. Sex Dev. 2016;10(1):45–57.
24. Skvortsova K, Iovino N, Bogdanović O. Functions and mechanisms of epigenetic inheritance in animals. Nat Rev Mol Cell Biol. 2018;19(12):774–90.
25. Agents stimulating gonadal function in the human. Report of a WHO scientific group. World Health Organ Tech Rep Ser. 1973;514:1–30.
26. Lichtenstein P, Holm NV, Verkasalo PK, et al. Analyses of cohorts of twins from Sweden, Denmark, and Finland. Medicine. 2000;343(2):78–85.
27. United States Environmental Protection Agency. TSCA chemical substance inventory. 2020. https://www.epa.gov/tsca-inventory.
28. Walsh BS, Parratt SR, Hoffmann AA, et al. The impact of climate change on fertility. Trends Ecol Evol. 2019;34(3):249–59.
29. Moritz C, Agudo R. The future of species under climate change: resilience or decline? Science. 2013;341(6145):504–8.

30. Intergovermental Panel on Climate Change. Climate Change. Impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. 2014. Cambridge, UK: Cambridge University Press; 2014. p. 1132.
31. Fuller A, Maloney SK, Blache D, et al. Endocrine and metabolic consequences of climate change for terrestrial mammals. Physiology. 2018;33(3):170–81.
32. Mckinley MJ, Davide M, Pennington GL, et al. Integrating competing demands of osmoregulatory and thermoregulatory homeostasis. Curr Opin Endocr Metab Res. 2020;11:9–14.
33. Roth Z. Effect of heat stress on reproduction in dairy cows—insights into the cellular and molecular responses of the oocyte. Ann Rev Anim Biosci. 2017;5(1):151–170.
34. Walker WH, Meléndezernández OH, Nelson RJ, et al. Global climate change and invariable photoperiods: a mismatch that jeopardizes animal fitness. Ecol Evol. 2019;9:5747.
35. Zatsepin I, Verger P, Robert-Gnansia E, et al. Down syndrome time-clustering in January 1987 in Belarus: link with the Chernobyl accident? Reprod. Toxicology. 2007;24:289–95.
36. Spitz DR, Azzam EI, Li JJ, Gius D. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology. Cancer Metas Rev. 2004;23:311–22.
37. Desouky O, Ding N, Zhou G. Targeted and non-targeted effects of ionizing radiation. J Radiat Res Appl Sci. 2015;8(2):247–54.
38. Sage E, Shikazono N. Radiation-induced clustered DNA lesions: repair and mutagenesis. Free Radic Biol Med. 2017;107:125–35.
39. Kam WY, Banati RB. The effects of ionizing radiation on mitochondria. Free Radic Biol Med. 2013;65(4):607–19.
40. van Dorp W, Haupt R, Anderson RA, et al. Reproductive function and outcomes in female survivors of childhood, adolescent, and young adult Cancer: A review. J Clin Oncol. 2018;36:2169–80.
41. Sapmaz-Metin M. The role of ionizing radiation on ovulation rate and oocyte morphology in mouse. Acta Biol Hung. 2014;65(1):27.
42. Damewood MD, Grochow LB. Prospects for fertility after chemotherapy or radiation for neoplastic disease. Fertil Steril. 1986;45:443–59.
43. Wallace WHB, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod. 2003;18(1):1.
44. Nicholas S, Chen L, Choflet A. Pelvic radiation and Normal tissue toxicity. Semin Radiat Oncol. 2017;27(4):358–69.
45. Byrne J, Fears TR, Mills JL, et al. Fertility in women treated with cranial radiotherapy for childhood acute lymphoblastic leukemia. Pediatr. Blood. Cancer. 2004;42:589–97.
46. Hardell L. World Health Organization, radiofrequency radiation and health-a hard nut to crack. Int J Oncol. 2017;51(2):405–13.
47. Cermak AMM, Pavicic I, Trosic I. Oxidative stress response in SH-SY5Y cells exposed to short-term 1800MHz radiofrequency radiation. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2018;53(2):132–8.
48. Lu J, Wang Z, Cao J, et al. A novel and compact review on the role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2018;16(1):80.
49. Sifakis S, Androutsopoulos VP, Tsatsakis AM, et al. Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. Environ Toxicol Pharmacol. 2017;51:56–70.
50. Gore AC, Chappell VA, Fenton SE, et al. EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr Rev. 2015;6:6.
51. Darbre PD. The history of endocrine-disrupting chemicals. Curr Opin Endocr Metab Res. 2019;7:26–33.
52. Ge W, Li L, Dyc PW. Establishment and depletion of the ovarian reserve: physiology and impact of environmental chemicals. Cell Mol Life Sci. 2019;76(9):1729–46.
53. Patel S. Fragrance compounds: the wolves in sheep's clothings. Med Hypotheses. 2017;102:106–11.

54. Solecki R, Kortenkamp A, Bergman Å, et al. Scientific principles for the identification of endocrine-disrupting chemicals: a consensus statement. Arch Toxicol. 2017;91(2):1001–6.
55. Machtinger R, Orvieto R. Bisphenol A, oocyte maturation, implantation, and IVF outcome: review of animal and human data. Reprod Biomed Online. 2014;29(4):404–10.
56. Karavan JR, Pepling ME. Effects of estrogenic compounds on neonatal oocyte development. Reprod Toxicol. 2012;34(1):51–6.
57. Hunt PA, Lawson C, Gieske M, et al. Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey. Proc Natl Acad Sci U S A. 2012;109(43):17525–30.
58. Vabre P, Gatimel N, Moreau J, et al. Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. Environ Health. 2017;16:37.
59. Gamez JM, Penalba R, Cardoso N, et al. Exposure to a low dose of bisphenol A impairs pituitary-ovarian axis in prepubertal rats effects on early folliculogenesis. Environ Toxicol Pharmacol. 2015;39(1):9–15.
60. Catherine V, Mhaouty-Kodja S, René H, et al. Evidence-based adverse outcome pathway approach for the identification of BPA as an endocrine disruptor in relation to its effect on the estrous cycle. Mol Cell Endocrinol. 2018;475:10–28.
61. Ziv-Gal A, et al. Evidence for bisphenol A-induced female infertility: a review (2007–2016). Fertil Steril. 2016;106(4):827–56.
62. Shen J, Kang Q, Mao Y, et al. Urinary bisphenol A concentration is correlated with poorer oocyte retrieval and embryo implantation outcomes in patients with tubal factor infertility undergoing in vitro fertilisation. Ecotoxicol Environ Saf. 2020;109816:187.
63. Kawa IA, Masood A, Ganie MA, et al. Bisphenol A (BPA) acts as an endocrine disruptor in women with Polycystic Ovary Syndrome: Hormonal and metabolic evaluation. Obesity Med. 2019;2019:100090.
64. Akgül S, Sur Ü, Düzçeker Y, et al. Bisphenol A and phthalate levels in adolescents with polycystic ovary syndrome. Gynecol Endocrinol. 2019;35(12):1–4.
65. Milanović M, Milošević N, Sudji J, et al. Can environmental pollutant bisphenol A increase metabolic risk in polycystic ovary syndrome? Clin Chim Acta. 2020;507:257–63.
66. Wang W, Hafner KS, Flaws JA. In utero bisphenol A exposure disrupts germ cell nest breakdown and reduces fertility with age in the mouse. Toxicol Appl Pharmacol. 2014;276:157–64.
67. Zhang T, Li L, Qin XS, et al. Di-(2-ethylhexyl) phthalate and bisphenol A exposure impairs mouse primordial follicle assembly in vitro. Environ Mol Mutagen. 2014;55:343–53.
68. Acuna-Hernández DG, Arreola-Mendoza L, et al. Bisphenol A alters oocyte maturation by prematurely closing gap junctions in the cumulus cell-oocyte complex. Toxicol Appl Pharmacol. 2018;344:13–22.
69. Murata M, Kang JH. Bisphenol A (BPA) and cell signaling pathways. Biotechnol Adv. 2018;36(1):311–27.
70. Moyer B, Hixon ML. Reproductive effects in F1 adult females exposed in utero to moderate to high doses of mono-2-ethylhexylphthalate (MEHP). Reprod Toxicol. 2012;34:43–50.
71. Zhang XF, Zhang T, Han Z, et al. Transgenerational inheritance of ovarian development deficiency induced by maternal diethylhexyl phthalate exposure. Reprod Fertil Dev. 2015;27:1213–21.
72. Niermann S, Rattan S, Brehm E, et al. Prenatal exposure to di-(2- ethylhexyl) phthalate (DEHP) affects reproductive outcomes in female mice. Reprod Toxicol. 2015;53:23–32.
73. Catheryne Chiang Lily R, Borkowski LG. Exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate during adulthood disrupts hormones and ovarian folliculogenesis throughout the prime reproductive life of the mouse. Toxicol Appl Pharmacol. 2020;393:114952.
74. Cao M, Pan W, Shen X, et al. Urinary levels of phthalate metabolites in women associated with risk of premature ovarian failure and reproductive hormones. Chemosphere. 2020;242:125206.

75. Meling DD, Warner GR, Szumski JR. The effects of a phthalate metabolite mixture on antral follicle growth and sex steroid synthesis in mice. Toxicol Appl Pharmacol. 2020;114875:388.
76. Bethsass J, Colangelo A. European Union bans atrazine, while the United States negotiates continued use. Int J Occup Environ Health. 2006;12(3):260–7.
77. Cragin LA, Kesner JS, Bachand AM, et al. Menstrual cycle characteristics and reproductive hormone levels in women exposed to atrazine in drinking water. Environ Res. 2011;111 (8):1293–301.
78. Samardzija D, Pogrmic-Majkic K, Fa S, et al. Atrazine blocks ovulation via suppression of Lhr and Cyp19a1 mRNA and estradiol secretion in immature gonadotropin-treated rats. Reprod Toxicol. 2016;61:10–8.
79. Pogrmic-Majkic K, Samardzija D, Fa S, et al. Atrazine enhances progesterone production through activation of multiple signaling pathways in FSH-stimulated rat Granulosa cells: evidence for premature Luteinization. Biol Reprod. 2014;91(5):124.
80. Pogrmic-Majkic K, Samardzija D, Stojkov-Mimic N, et al. Atrazine suppresses FSH-induced steroidogenesis and LH-dependent expression of ovulatory genes through PDE-cAMP signaling pathway in human cumulus granulosa cells. Mol Cell Endocrinol. 2017;461:79–88.
81. Palmirotta R, Lovero D, Silvestris E. Nutrition and female fertility: an interdependent correlation. Front Endocrinol. 2019;10:346.
82. Hart RJ. Physiological aspects of female fertility: role of the environment, modern lifestyle, and genetics. Physiol Rev. 2016;96(3):873–909.
83. Boutari C, Pappas PD, Mintziori G, et al. The effect of underweight on female and male reproduction. Metabolism. 2020;107:154229.
84. Yu-Han C, Chavarro JE, Irene S. Diet and female fertility: doctor, what should I eat? Fertil Steril. 2018;110(4):560–9.
85. Sharma R, Kelly R. Biedenharn. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol. 2013;11(1):1–15.
86. Chavarro JE, Rich-Edwards JW, Rosner BA, et al. Diet and lifestyle in the prevention of ovulatory disorder infertility. Obstet Gynecol. 2007;110(5):1050–8.
87. Gaskins AJ, Chavarro JE. Diet and fertility: a review. Am J Obstet Gynecol. 2018;218 (4):379–89.
88. Broughton DE, Moley KH. Obesity and female infertility: potential mediators of obesity's impact. Fertil Steril. 2017;107(4):840–7.
89. Bazzano MV, Paz DA, et al. Obesity alters the ovarian glucidic homeostasis disrupting the reproductive outcome of female rats. J Nutr Biochem. 2017;42:194–202.
90. Mintziori G, Nigdelis MP, Mathew H, et al. The effect of excess body fat on female and male reproduction. Metabolism: Clin Exp. 2020;107:154193.
91. Leshan RL, Pfaff DW. The hypothalamic ventral pre mammillary nucleus: a key site in leptin's regulation of reproduction. J Chem Neuroanat. 2014;61–62:239–47.
92. Silvestris E, De Pergola G, Rosania R, et al. Obesity as disruptor of the female fertility. Reprod Biol Endocrinol. 2018;16(1):22.
93. Woodward A, Klonizakis M, Broom D. Exercise and polycystic ovary syndrome. Adv Exp Med Biol. 2020;1228:123–36.
94. Clark AM, Thornley B, Tomlinson L, et al. Weight loss results in significant improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod. 1998;13:1502–5.
95. Glueck CJ, Goldenberg N. Characteristics of obesity in polycystic ovary syndrome: etiology, treatment, and genetics. Metabolism. 2019;92:108–20.
96. Benham JL, Yamamoto JM, Friedenreich CM, et al. Role of exercise training in polycystic ovary syndrome: a systematic review and meta-analysis. Clin Obesity. 2018;8(4):275–84.
97. Hakimi O, Cameron LC. Effect of exercise on ovulation: a systematic review. Sports Med. 2017;47(8):1555–67.
98. Nose-Ogura S, Harada M, Hiraike O, et al. Management of the female athlete triad. J Obstet Gynaecol Res. 2018;44(6):1007–14.

99. De Souza MJ, Koltun KJ, Etter CV, et al. Current status of the female athlete triad: update and future directions. Curr Osteoporos Rep. 2017;15(6):577–87.
100. Shiverick KT. Cigarette smoking and reproductive and developmental toxicity. In: Reproductive and developmental toxicology. Amsterdam: Elsevier Inc; 2017.
101. Alan P, Kristin B, Samantha B, et al. Smoking and infertility: a committee opinion. Fertil Steril. 2018;110(4):611–8.
102. Budani MC, Tiboni GM. Ovotoxicity of cigarette smoke: a systematic review of the literature. Reprod Toxicol. 2017;72:164–81.
103. Sobinoff AP, Beckett EL, Jarnicki AG, et al. Scrambled and fried: cigarette smoke exposure causes antral follicle destruction and oocyte dysfunction through oxidative stress. Toxicol Appl Pharmacol. 2013;271(2):156–67.
104. Cho-Won K, Ryeo-Eun G, Kyung-A H, et al. Effects of cigarette smoke extracts on apoptosis and oxidative stress in two models of ovarian cancer in vitro. Toxicol In Vitro.
105. Grodstein F, Goldman MB, Cramer DW. Infertility in women and moderate alcohol use. Am J Public Health. 1994;84:1429–32.
106. Van Heertum K, Rossi B. Alcohol and fertility: how much is too much? Fertil Res Pract. 2017;3(1):10.
107. Ilnitsky S, Uum SV. Marijuana and fertility. Can Med Assoc J. 2019;191(23):638.
108. Brents LK. Marijuana, the Endocannabinoid system and the female reproductive system. Yale J Biol Med. 2016;89(2):175–91.
109. Drake LR, Scott PJH. DARK classics in chemical neuroscience: cocaine. ACS Chem Neurosci. 2018;9(10):2358–72.
110. Thyer AC, King TS, Moreno AC, et al. Cocaine impairs ovarian response to exogenous gonadotropins in nonhuman primates. J Soc Gynecol Investig. 2001;8:358–62.
111. Valsamakis G, Chrousos G, Mastorakos G. Stress, female reproduction and pregnancy. Psychoneuroendocrinology. 2019;100:48–57.
112. Sominsky L, Hodgson DM, McLaughlin EA, et al. Linking stress and infertility. Endocr Rev. 2017;38:432–67.
113. Reindollar RH, Novak M, Tho SP, McDonough PG. Adult onset amenorrhea: a study of 262 patients. Am J Obstet Gynecol. 1986;155:531–43.
114. Berga SL. Stress-induced anovulation. In: Stress: physiology, biochemistry, and pathology. Amsterdam: Elsevier; 2019.
115. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Clin Neurosci. 2006;8(4):383–95.
116. Mccosh RB, Breen KM, Kauffman AS. Neural and endocrine mechanisms underlying stress-induced suppression of pulsatile LH secretion. Mol Cell Endocrinol. 2019;498:110579.
117. Kala M, Nivsarkar M. Role of cortisol and superoxide dismutase in psychological stress induced anovulation. Gen Comp Endocrinol. 2015;225:117–24.
118. Sanders KM, Kawwass JF, Loucks T, et al. Heightened cortisol response to exercise challenge in women with functional hypothalamic amenorrhea. Am J Obstet Gynecol. 2018;218:230.
119. Michopoulos V, Mancini F, Loucks TL, et al. Neuro-endocrine recovery initiated by cognitive behavioral therapy in women with functional hypothalamic amenorrhea: a randomized, controlled trial. Fertil Steril. 2013;99(7):2084–91.

Chapter 6
Effects of Cigarette Smoking on Preimplantation Embryo Development

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Abstract In this chapter, we first gave a brief introduction to the detriments of cigarette smoking, with an emphasis on its adverse effects on female reproductive health. Then, we outlined recent advances about the impacts of cigarette smoke on preimplantation embryo development. Additionally, toxicities of cadmium and benzo(a)pyrene (BaP) at this specific developmental window were also discussed, to illustrate the potential mechanisms involved in cigarette smoke-associated embryotoxicity. Finally, we provide an overview of the issues to be solved in the future research. Further studies about the molecular mechanism of cigarette smoking-associated female infertility may provide vital insights into developing new interventions for the women smokers and thus improving their reproductive outcomes.

Keywords Cigarette smoke · Female reproduction · Preimplantation embryo development · Cadmium · BaP · Infertility

6.1 Introduction

The fact that there is still over 1 billion smokers worldwide suggests that major gaps remain, undoubtedly, in meeting the requirements of the World Health Organization (WHO) Framework Convention on Tobacco Control, although some countries have made a great progress since its adoption in 2003 [1]. Thus, much more enhanced measures and great efforts should be made for tobacco control, since cigarette

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smoking can cause devastating impacts on every aspect of human health. For example, according to a recent report, over 11.5% of global deaths (about six million every year) were attributable to cigarette smoking worldwide [2]. Cigarette smokers had a twice mortality rate than the never-smoker, predominately due to chronic degenerative respiratory diseases, lung cancer, and cardiovascular diseases [3]. Additionally, cigarette smoking can markedly jeopardize the immune system, weakening the host's ability to defend against microbial infection and harmful agents, and thus worsening respiratory pathologies such as asthma and tuberculosis [4]. Lastly, human infertility that has been a global problem and occurs in approximately 15% of reproductive-age couples [5] is also included in the list of non-lethal health risks induced by cigarette smoke. As estimated, about 13% of clinical infertility cases can be attributed to cigarette smoking, linking to higher risk of spontaneous abortion in both natural and assisted conception cycles [6–9]. Therefore, it is of great importance to illuminate the molecular mechanisms underlying the smoking-associated reproductive toxicology.

Although increasing scientific and clinical evidence has suggested that cigarette smoke has extremely adverse effects on the female reproductive health, the mechanisms underpinning are not fully understood, since the female reproductive system and process are tremendously complicated, involving delicate coordination of multiple organs and tissues. In human, a successful pregnancy and delivery depends on success of all the stages in the female reproductive process, including folliculogenesis in ovary, egg fertilization, preimplantation development and embryo transport in reproductive tracts, embryo implantation in uterus, and subsequent intrauterine fetus development nourished and supported by placenta (Chap. 2). The process of the assisted reproductive technologies (ART) that have been widely used for human infertility treatment [10] is yet slightly different. After ovarian stimulation and oocyte retrieve, ART routinely involves a series of in vitro embryonic manipulation that are carried out in laboratory for about 3–6 days, before embryos are transferred back toward the patients' uterus. This process corresponds to the window of human preimplantation development in vivo, which is crucial for a successful pregnancy and delivery to term. During the first 3 days, after fusion of the egg with sperm, the resulting totipotent zygote initiate preimplantation development, followed by a chain of cleavages that are simultaneous with numerous crucial events, including the elimination of maternal mRNAs and proteins, epigenetic reprogramming, as well as embryonic genome activation. Subsequently, the embryo undergoes compaction to form a morula and then develops into a blastocyst. The embryo at blastocyst stage comprises two main lineages of cells: the inner cell mass (ICM) that is the most important source for fetus development and the outer trophectoderm (TE) that will develop into extra embryonic tissues involved in the placenta [11, 12].

Given the critical role of preimplantation development in female reproduction, we will focus on the harmful effects of cigarette smoke on preimplantation embryo development in this chapter. We believe that full elucidation of this issue will provide insights into our understanding of cigarette-induced clinical infertility.

6.2 Effects of Cigarette Smoking on Female Reproduction

Cigarette smoke toxicants and/or their metabolites have been detected in multiple tissues or fluids of pregnant women, including the uterus, placenta, fetal cord blood, maternal peripheral blood, and breast milk, suggesting that through the circulatory system, cigarette components eventually are able to reach their target tissues and/or organs, to exert a detrimental effect on reproductive physiology [13–17]. The past few decades have seen a major leap forward in understanding the devastating impacts of cigarette smoking on human reproduction, based on combinatorial data from human clinic and animal models in vivo and in vitro.

Firstly, since the production and quality of oocytes is an important starting point for female reproduction, smoking can affect fertility at the source. Direct or indirect exposure to environmental toxins including cigarette smoke can affect the ovarian function in many ways, leading to impaired steroidogenesis and follicular development, follicles loss [18–22], and poor oocyte quality [23, 24]. For example, abnormal endocrine characterized by higher level of testosterone and follicle-stimulating hormone (FSH) was found in female smokers [21, 22]. Also, cigarette smoking is associated with aneuploidy, because exposure to the harmful ingredients in smoking can induce meiotic spindle disturbance and chromosomal misalignment, leading to production of oocytes with abnormal chromosome numbers. In a mouse model, despite of the similar numbers and maturation rates, 24% of the oocytes recovered form mice directly exposed to cigarette smoke had severe anomalies in spindle structure or chromosomal alignment, compared to merely 2% in non-smoking mice, mainly due to shorter spindle length and wider spindle equators [23]. Poor quality indicated by disorganized microfilaments during in vitro meiotic maturation was also found in bovine oocytes exposed to nicotine, accompanied with decreased maturation rates in a dose-dependent manner, ranging from about 90% in the non-treated group to just 15% in the group exposed to nicotine of 6 nM [24].

What is worse, declined quality of these oocytes compromised subsequent parthenogenetic development, with only about 5% of the resultant embryos developing to blastocysts that were almost aneuploid and hypogenetic with obviously decreased cell number [24]. This indicated that cigarette smoking might also impair the subsequent development following ovulation. In a clinical In Vitro Fertilization (IVF) program, female smokers had a notably lower fertilization rate, indicated by approximately 10% more non-fertilizated oocytes (20.1%) than that in non-smokers (10.8%) [25]. Using mammalian models, we and others demonstrated that exposure to cigarette smoke lead to compromised preimplantation embryonic development [26–30], which will be discussed later in detail.

In addition, a significantly lower pregnancy rate was also observed in those heavy-smoking recipients in the cycles of oocyte donation when compared to non-heavy smokers (34% VS 52%, respectively) [31], suggesting that cigarette smoking can affect embryo implantation. Successful implantation of embryos requires the establishment of uterine receptivity, in which the endometrium plays a major role. A previous study based on human endometrial stromal cells reported that

cadmium, a toxic metal with a significantly high level in cigarette, could induce early decidualization, and thus disrupt endometrial function [32]. This may be a persuasive explain for the lower pregnancy rates in heavy smokers [31], and implies a potential mechanism involved in cigarette smoke-induced implantation failure.

Furthermore, recent epigenetic studies indicate that offspring physiological abnormalities can result from unhealthy parental lifestyles including cigarette smoking, although the mechanism remains to be clearly elucidated [33–35]. Both maternal smoking and indirect exposure to cigarette smoke during gestation can either compromise prenatal intrauterine development directly, by jeopardizing organogenesis and delaying the fetal growth [35–37] or exert long-term consequences on the offspring, till their adulthood [35, 38, 39].

In summary, cigarette smoke can target almost all stages during female reproduction [40]. Various reproductive diseases can result from smoking-induced dysfunction at any corresponding stage of these processes, rendering to decreased female fecundity and even infertility.

6.3 Research Models for Preimplantation Embryo Development

As aforementioned, cigarette smoke can target the whole process during female reproduction, including preimplantation stage [26, 27]. Therefore, full elucidation of the effects of smoking on human preimplantation embryonic development, will provide significant insights into our understanding of the pathogenesis of cigarette-associated female sterility, and may thus enable development of new interventions to improve the clinical reproductive outcome, especially for those female smokers in IVF program [31].

However, investigations involving early human embryo are always limited, for the lack of these precious materials, and potential ethical concerns on using such kind of materials. Since animal studies can control experimental conditions rigorously and exclude potentially miscellaneous variables that may present merely in the clinical IVF process, relevant studies can usually provide adequately scientific and reliable information, reflecting results in human to some extent. Therefore, as an alternative, there is of great demand to establish appropriate animal models that are evolutionarily similar to humans, in terms of the anatomy, physiology, as well as the embryology and reproductive biology.

The rodent, like mice and rats, have been widely used, for not only their similarities with human in many terms as aforementioned, but also their strong capacity and short cycle of reproduction, which make them an ideal research model for early human embryonic development. Both in vitro and in vivo toxicity studies conducted in these animals [26, 27], have provided a plenty of experimental data to recapitulate the characteristics of smoking-induced embryotoxicity, as well as potential mechanisms underlying cigarette-associated human infertility. Besides, the

embryonic stem cells (ESCs) derived from the ICM in preimplantation blastocyst, has been widely utilized as a superior model alternative to laboratory animals to determinate the toxicity of environmental contaminants and chemicals on early mammalian development, aiming to reduce the animal use and thus improve the animal welfare [28, 41, 42].

Hereafter, we will highlight recent advances in the effects of cigarette smoking on preimplantation embryonic development, mainly based on these in vitro and in vivo studies.

6.4 Effects of Cigarette Smoking on Preimplantation Embryo Development

Constituents of cigarette smoke or their metabolites, including nicotine, cadmium, and benzo(a)pyrene (BaP), have been identified for many years in cleavage embryo proper, uterine endometrium, and cervical fluid [43–46], suggesting a detrimental environment around preimplantation embryos. In human, since it is quite difficult to detect the effects of cigarette smoke on preimplantation development in vivo directly, data about this particular process are obtained almost only from the epidemiological and clinical statistical studies, and remain poor and controversial.

Shiloh et al. reported that active and passive cigarette smoking, classified according to self-reported smoking habits, increased the zona pellucida thickness of both the oocytes (approximately 20μm in smokers VS 15μm in non-smokers) and preimplantation embryos (ranging from 18 to 20μm in smokers VS 15μm in non-smokers approximately) [47]. This result indicated that cigarette smoking might affect the fertilization and embryo implantation, two critical processes that involve zona pellucida. But it remained to be confirmed, since no data were available in this study regarding the corresponding fertilization, implantation, and pregnancy rates between different groups. A retrospective clinical study showed that there were no obvious difference between the smokers and non-smokers in embryo morphology and quality, as well as the cleavage and clinical pregnancy rate, but a lower fertilization rate in smokers, when compared to the non-smoking counterparts (approximately 78.2% VS 85.7%, $P < 0.01$ by Chi-square) [25]. Despite of similar observation for embryo quality, this was conflicting to a previous report [48], in which the authors found strikingly decreased implantation and pregnancy rates of mainstream (12.0% and 19.4%, respectively) and sidestream (12.6% and 20.0%, respectively) smoke-exposed women, compared to the non-smokers (25.0% and 48.3%, respectively, for implantation and pregnancy rates). Thus, vast arrays of more comprehensive data are of great need to clearly elucidate the effects of cigarette smoke on human preimplantation development in the future.

Fortunately, during the past decades, animal studies have provided a plenty of experimental data to promote our understanding of cigarette smoke-induced toxicity on preimplantation development. Hassa et al. found that the rates of fertilization and

cleavage were significantly lower in cigarette smoke-exposed mice (20.6% and 17.2%, respectively) than the controls (85% and 75%, respectively) [49]. Consistently, we showed that mice chronically exposed to cigarette smoke in vivo before mating, exhibited increased egg fragmentation and delayed fertilization [26]. When these in vivo fertilized embryos from exposed mice were cultured in vitro, we found that cleaved embryos were significantly reduced in mice exposed to cigarette smoke condensate (CSC) that are made from cigarettes, compared with controls (73.3% VS 87.7%, $P < 0.05$). Additionally, the rate of these cleaved embryos reaching the blastocyst stage from mice exposed to CSC (68.1%) and cigarette smoke (71.4%) were obviously less than that of control subjects (80.2%, $P < 0.05$). Simultaneously, we evaluated the effects of acute exposure on this specific window of embryo development, by using the whole animal exposure model after mating and fertilization, and found similar observation [26]. Furthermore, by using CSC, deleterious effects of acute or chronic smoke exposure on early development were also evaluated in early mouse embryos in vitro [27], as well as in mouse embryonic stem cells (mESCs) that plays a vital role in subsequent fetal development following embryo implantation [28], confirming the in vivo results [26]. Together, these in vitro and in vivo results mentioned above allow us to conclude that cigarette smoke compromises the preimplantation embryo development.

However, the mechanisms underlying cigarette-induced embryotoxicity and thus associated sterility are poorly understood. This is mainly due to the difficulty to distinguish the effective toxicants that are responsible for the reproductive toxic effects from tobacco and/or smoke mixture that consists of over 4000 chemical components [50]. What is more, the toxicity of great majority of cigarette chemicals are still unknown. In light of the consideration that effects of cigarette smoke may be a result as the combination of the toxicity of various compounds it contains, it will be partial to utilize individual constituents of cigarette smoke to evaluate the overall effects of smoking. Nevertheless, relevant studies evaluating the deleterious effects of individual component of cigarette smoke, such as nicotine [24, 29], cadmium [27, 28, 32, 51], and BaP [30, 48, 52–54], are informative and promotes our understanding of the mechanisms involving in cigarette-induced toxicity. Herein, cadmium and BaP, as examples, will be discussed in detail to illuminate the effects and potential mechanisms of cigarette components on mouse preimplantation embryo development.

6.5 Effects of Cadmium on Preimplantation Embryo Development

The heavy metal cadmium is a toxic abundant component of cigarette (about 1.0–2.0μg per cigarette), up to 10% of which can be absorbed by human through the smoke after burning [55]. Inhaled cadmium was detected in blood and reproductive system, including female follicular fluids and male seminal plasma, with a

remarkably higher exposure level in heavy smokers [46, 55–58]. Embryonic degeneration with necrosis appearance was found in mouse embryos exposed to high dose of cadmium, at 5–10μg/mL [51]. Also, a previous study conducted on rabbit also showed that cadmium-induced necrosis inside the ICM cells at blastocysts, characterized with cytoplasmic vacuoles and residual bodies [59]. These data suggested that cadmium could negatively affect the preimplantation embryonic development, at least at high levels of exposure.

Consistently, we showed that embryos exposed to cadmium in vitro demonstrated time- and dosage-dependent developmental arrest and death [27]. Mouse zygotes chronically exposed for 96 h to cadmium at 20μM, a relatively lower level than that in previous study [51], had a comparable cleavage rate to the control without exposure (90%), whereas all the zygotes failed to cleave when exposed for merely 20 h to cadmium at 100μM. Furthermore, all the cleaved embryos exposed to 20μM cadmium arrested prior to morula stages, and these arrested embryos underwent cell death eventually, which might be attributable to increased reactive oxygen species (ROS) [27]. Although up to 67% of embryos exposed to 5μM cadmium, a much lower exposure level, could develop into blastocysts, significantly lower than the controls (89%), these embryos showed significantly decreased embryo qualities, indicated by reduced total cell number, increased telomere loss and chromosome fusion, as well as shorter telomere length.

Telomeres, the very end structure of chromosomes, can maintain genome stability by protecting the chromosomes from recombination and fusions, while are susceptible to oxidative damage [28, 60]. The roles of telomere function in early embryo development and ESCs pluripotency have been well documented [61, 62]. Since no blastocysts was available at the exposure level of 20μM, we tested the effects of cadmium on the mouse ESCs functions. Acute exposure to cadmium at 20μM leads to reduced ESCs pluripotency and even immediate cell death, whereas chronic exposure to the same level of cadmium results in DNA damage accumulation and telomere shortening [28]. These data verified that cadmium negatively affected the preimplantation blastocyst development, especially for the ICM that is the source of ESCs, further confirming the previous result [59].

Remarkably, these cadmium-induced damages to the embryo or ESCs, including decreased embryonic cleavage and developmental potential, reduced ESCs pluripotency, and shorter telomere length, as well as increased DNA damage and dysfunctional telomeres, could be alleviated effectively by co-treatment with appropriate concentrations of the antioxidant, N-Acety-L-Cysteine (NAC) [27, 28], suggesting that increased ROS may partially account for the cadmium-induced embryotoxicity in mechanism. This may provide an additional explain for the lower implantation rate in those who are heavy smoker with high exposure level to cadmium [31], and thus a potentially new intervention strategy for human infertility.

6.6 Effects of BaP on Preimplantation Embryo Development

BaP, a proven carcinogen belonging to the PAHs family and also an ubiquitous environmental pollutant, is present in secondhand smoke at relatively high levels, almost 10 times higher than mainstream smoke [63]. BaP and its metabolic derivatives have been found in follicular fluid and embryo proper, suggesting its potential involvement in reproduction [20, 43, 53]. A previous study [53] showing that the level of BaP in the follicular fluid of female mainstream smokers who did not conceive (1.79 $\pm$ 0.03 ng/mL) was significantly higher than that of those non-smokers who achieved a pregnancy (0.08 $\pm$ 0.03 ng/mL), implied that BaP might account for smoking-associated abortion and female infertility. However, effects of BaP on preimplantation development remain to be explored, especially at the level representing human exposure in ovarian follicular fluid and serum [20], although BaP-induced toxicity in multiple processes of female reproduction has been well described, leading to ovarian, uterine and placental dysfunctions [20, 52, 54, 64].

Hence we studied the effects of direct BaP exposure on preimplantation development of mouse zygote in vitro [30]. Initially, neither the morphology nor the embryo cleavage and blastocyst formation was found to be significantly different between exposed zygotes and unexposed controls. But, just for about 4 h and even at 5 nM, approximately the physiological level of human in vivo exposure, BaP exposure had obviously increased the ROS levels (34.5% more than the control), which was implicated in BaP toxicology in previous reports [65, 66]. Then, given that ROS can destroy cellular organelles and macromolecules, including mitochondria, proteins, as well as DNA [67], and that BaP and its metabolites can target DNA to form BaP-DNA adducts, which can impede the process of DNA replication and thereby lead to replication errors [68], we tested whether increased ROS could lead to poor embryo quality, by terminal-deoxynucleoitidyl transferase mediated nick end labeling (TUNEL) and telomere dysfunction induced foci (TIF) assay to detect cell apoptosis and DNA damage in blastocysts developed from BaP-exposed zygotes [30]. As a result, BaP exposure caused increased apoptosis (with averagely 8.6% $\pm$ 1.6% and 7.6% $\pm$ 1.2% apoptotic cells per embryo exposed to 5 nM and 50 nM BaP, respectively, when compared to 3.7% $\pm$ 0.7% in control; $P < 0.05$), and induced serious DNA damage in the whole genome, as well as in telomeres. Similarly, severe genomic and telomeric DNA damage are also found in BaP-exposed mESCs [30], suggesting that BaP might also induce DNA damage in the ICM proper and thus reduce their developmental potential following implantation.

Moreover, poor quality was also indicated by fewer Oct4 (26.8% VS 24.4%, $P < 0.05$) and Nanog (14.1% VS 17.4% in control, $P < 0.001$) positive ICM cells in late blastocysts from BaP-exposed zygotes [30]. The Oct4 and Nanog protein, respectively, are specific marker of ICM and epiblast (EPI), a later derivative from ICM, and *Oct4*- or *Nanog*-deficient embryos exhibit post-implantation

developmental defects for the incapability to generate functional ICM [69–71]. Therefore, reduced number and poor quality of ICM, which resulted from BaP exposure, might affect its potential to generate functional EPI and primitive endoderm (PrE) lineages, and thus compromise subsequent fetal development, and potentially increase the risk of early pregnancy loss (EPL) [53].

Whereas, how does BaP work? Previous studies have showed that effects of PAHs, including BaP, might be mediated via the aryl hydrocarbon receptor (AhR), and expression of the AhR during early embryonic development has also been shown [72, 73]. For the mechanism involving premature ovarian failure, PAHs activation of the AhR, has been described to induce apoptosis by promoting Bax expression [74, 75]. So, it was reasonable to suspect that BaP also activated AhR pathway to induce apoptosis in exposed embryos. However, it remains to be well elucidated whether and how BaP act via AhR pathway to alter gene expression programs, disrupting the intracellular antioxidant defense and thus increasing ROS levels.

6.7 Conclusions and Future Prospects

Based on both basic and clinical studies, numerous progresses have been made, suggesting that cigarette smoke proper and/or its constituents can disrupt preimplantation embryo development, rendering to poor quality of subsequent embryos, and thus contributing to smoke related pregnancy loss and female infertility. However, further research is needed to fully dissect the following issues: (1) Molecular mechanisms underlying environmental agents induced embryotoxicity. The rapid advance of next-generation sequencing technologies provide useful tools for comprehensive profiling of the multiple omics dynamics in early human development [76, 77]. Whole-genome-sequencing (GWS) has also been reported to support exploration of the effects of environmental agents in cancer etiology [78]. The application of these techniques in the field of reproductive toxicology will provide more detailed information about the molecular mechanisms underlying the embryotoxicity of environmental agents, including cigarette smoke. (2) Prevention and interference for smoke-induced toxicity. Given the ROS involvement and that antioxidants, like resveratrol, melatonin, as well as N-Acety-L-Cysteine (NAC), have been found to alleviate the embryotoxicity of tobacco smoke and cadmium, and even delay reproductive aging [79–81], further exploration and discovery of natural or biosynthetic drugs that can antagonize the toxicity of smoke-exposed embryos, will be of great help for clinical prevention and treatment of smoking-induced infertility, although smoking cessation is always the first choice.

We believe that fully elucidation of these issues will provide a valuable resource for our understanding of the pathogenesis of cigarette-associated female sterility, with potential implications for human reproduction medicine.

References

1. Bilano V, Gilmour S, Moffiet T, d'Espaignet ET, Stevens GA, Commar A, Tuyl F, Hudson I, Shibuya K. Global trends and projections for tobacco use, 1990-2025: an analysis of smoking indicators from the WHO Comprehensive information Systems for Tobacco Control. Lancet. 2015;385(9972):966–76.
2. Collaborators GBDT. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990-2015: a systematic analysis from the Global Burden of Disease Study 2015. Lancet. 2017;389(10082):1885–906.
3. Chen Z, Peto R, Zhou M, Iona A, Smith M, Yang L, Guo Y, Chen Y, Bian Z, Lancaster G, Sherliker P, Pang S, Wang H, et al. Contrasting male and female trends in tobacco-attributed mortality in China: evidence from successive nationwide prospective cohort studies. Lancet. 2015;386(10002):1447–56.
4. Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. Nat Rev Immunol. 2009;9(5):377–84.
5. Jarow JP, Sharlip ID, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, Schlegel PN, Howards SS, Nehra A, Damewood MD, Overstreet JW, Sadovsky R, Male Infertility Best Practice Policy Committee of the American Urological Association I. Best practice policies for male infertility. J Urol. 2002;167(5):2138–44.
6. ASRM. Smoking and infertility. Fertil Steril. 2004;81(4):1181–6.
7. Hughes EG, Brennan BG. Does cigarette smoking impair natural or assisted fecundity? Fertil Steril. 1996;66(5):679–89.
8. Ness RB, Grisso JA, Hirschinger N, Markovic N, Shaw LM, Day NL, Kline J. Cocaine and tobacco use and the risk of spontaneous abortion. New Engl J Med. 1999;340(5):333–9.
9. Winter E, Wang J, Davies MJ, Norman R. Early pregnancy loss following assisted reproductive technology treatment. Hum Reprod. 2002;17(12):3220–3.
10. Mansour R, Ishihara O, Adamson GD, Dyer S, de Mouzon J, Nygren KG, Sullivan E, Zegers-Hochschild F. International Committee for Monitoring Assisted Reproductive Technologies world report: assisted reproductive technology 2006. Hum Reprod. 2014;29(7):1536–51.
11. Yan L, Yang M, Guo H, Yang L, Wu J, Li R, Liu P, Lian Y, Zheng X, Yan J, Huang J, Li M, Wu X, et al. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat Struct Mol Biol. 2013;20(9):1131–9.
12. Xue Z, Huang K, Cai C, Cai L, Jiang CY, Feng Y, Liu Z, Zeng Q, Cheng L, Sun YE, Liu JY, Horvath S, Fan G. Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. Nature. 2013;500(7464):593–7.
13. Madhavan ND, Naidu KA. Polycyclic aromatic hydrocarbons in placenta, maternal blood, umbilical cord blood and milk of Indian women. Hum Exp Toxicol. 1995;14(6):503–6.
14. McLachlan JA, Dames NM, Sieber SM, Fabro S. Accumulation of nicotine in the uterine fluid of the six-day pregnant rabbit. Fertil Steril. 1976;27(10):1204–13.
15. Paszkowski T. Concentration gradient of cotinine between blood serum and preovulatory follicular fluid. Ginekol Pol. 1998;69(12):1131–6.
16. Ptashekas J, Ciuniene E, Barkiene M, Zurlyte I, Jonauskas G, Sliachtic N, Babonas J, Jankeviciene R, Runkelyte J, Saltiene Z. Environmental and health monitoring in Lithuanian cities: exposure to heavy metals and benz(a)pyrene in Vilnius and Siauliai residents. J Environ Pathol Toxicol Oncol. 1996;15(2–4):135–41.
17. Staessen JA, Nawrot T, Hond ED, Thijs L, Fagard R, Hoppenbrouwers K, Koppen G, Nelen V, Schoeters G, Vanderschueren D, Van Hecke E, Verschaeve L, Vlietinck R, et al. Renal function, cytogenetic measurements, and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers. Lancet. 2001;357(9269):1660–9.
18. Sadeu JC, Foster WG. Effect of in vitro exposure to benzo[a]pyrene, a component of cigarette smoke, on folliculogenesis, steroidogenesis and oocyte nuclear maturation. Reprod Toxicol. 2011;31(4):402–8.

19. Tuttle AM, Stampfli M, Foster WG. Cigarette smoke causes follicle loss in mice ovaries at concentrations representative of human exposure. Hum Reprod. 2009;24(6):1452–9.
20. Neal MS, Zhu J, Holloway AC, Foster WG. Follicle growth is inhibited by benzo-[a]-pyrene, at concentrations representative of human exposure, in an isolated rat follicle culture assay. Hum Reprod. 2007;22(4):961–7.
21. Barbieri RL, Sluss PM, Powers RD, McShane PM, Vitonis A, Ginsburg E, Cramer DC. Association of body mass index, age, and cigarette smoking with serum testosterone levels in cycling women undergoing in vitro fertilization. Fertil Steril. 2005;83(2):302–8.
22. Cooper GS, Baird DD, Hulka BS, Weinberg CR, Savitz DA, Hughes CL Jr. Follicle-stimulating hormone concentrations in relation to active and passive smoking. Obstet Gynecol. 1995;85 (3):407–11.
23. Jennings PC, Merriman JA, Beckett EL, Hansbro PM, Jones KT. Increased zona pellucida thickness and meiotic spindle disruption in oocytes from cigarette smoking mice. Hum Reprod. 2011;26(4):878–84.
24. Liu Y, Li GP, White KL, Rickords LF, Sessions BR, Aston KI, Bunch TD. Nicotine alters bovine oocyte meiosis and affects subsequent embryonic development. Mol Reprod Dev. 2007;74(11):1473–82.
25. Gruber I, Just A, Birner M, Losch A. Effect of a woman's smoking status on oocyte, zygote, and day 3 pre-embryo quality in in vitro fertilization and embryo transfer program. Fertil Steril. 2008;90(4):1249–52.
26. Huang J, Okuka M, McLean M, Keefe DL, Liu L. Effects of cigarette smoke on fertilization and embryo development in vivo. Fertil Steril. 2009;92(4):1456–65.
27. Huang J, Okuka M, McLean M, Keefe DL, Liu L. Telomere susceptibility to cigarette smoke-induced oxidative damage and chromosomal instability of mouse embryos in vitro. Free Radic Biol Med. 2010;48(12):1663–76.
28. Huang JJ, Okuka M, Lu WS, Tsibris JCM, McLean MP, Keefe DL, Liu L. Telomere shortening and DNA damage of embryonic stem cells induced by cigarette smoke. Reprod Toxicol. 2013;35:89–95.
29. Liu Y, Li GP, Sessions BR, Rickords LF, White KL, Bunch TD. Nicotine induces multinuclear formation and causes aberrant embryonic development in bovine. Mol Reprod Dev. 2008;75 (5):801–9.
30. Zhan S, Zhang X, Cao S, Huang J. Benzo(a)pyrene disrupts mouse preimplantation embryo development. Fertil Steril. 2015;103(3):815–25.
31. Soares SR, Simon C, Remohi J, Pellicer A. Cigarette smoking affects uterine receptiveness. Hum Reprod. 2007;22(2):543–7.
32. Tsutsumi R, Hiroi H, Momoeda M, Hosokawa Y, Nakazawa F, Yano T, Tsutsumi O, Taketani Y. Induction of early decidualization by cadmium, a major contaminant of cigarette smoke. Fertil Steril. 2009;91(4 Suppl):1614–7.
33. Kaur G, Begum R, Thota S, Batra S. A systematic review of smoking-related epigenetic alterations. Arch Toxicol. 2019;93(10):2715–40.
34. Wu L, Lu Y, Jiao Y, Liu B, Li S, Li Y, Xing F, Chen D, Liu X, Zhao J, Xiong X, Gu Y, Lu J, et al. Paternal psychological stress reprograms hepatic gluconeogenesis in offspring. Cell Metab. 2016;23(4):735–43.
35. Stocks J, Hislop A, Sonnappa S. Early lung development: lifelong effect on respiratory health and disease. Lancet Respir Med. 2013;1(9):728–42.
36. de Souza MS, Lima PH, Sinzato YK, Rudge MV, Pereira OC, Damasceno DC. Effects of cigarette smoke exposure on pregnancy outcome and offspring of diabetic rats. Reprod Biomed Online. 2009;18(4):562–7.
37. Detmar J, Rennie MY, Whiteley KJ, Qu D, Taniuchi Y, Shang X, Casper RF, Adamson SL, Sled JG, Jurisicova A. Fetal growth restriction triggered by polycyclic aromatic hydrocarbons is associated with altered placental vasculature and AhR-dependent changes in cell death. Am J Physiol Endocrinol Metab. 2008;295(2):E519–30.

38. Zakarya R, Adcock I, Oliver BG. Epigenetic impacts of maternal tobacco and e-vapour exposure on the offspring lung. Clin Epigenetics. 2019;11(1):32.
39. Jurisicova A, Taniuchi A, Li H, Shang Y, Antenos M, Detmar J, Xu J, Matikainen T, Benito Hernandez A, Nunez G, Casper RF. Maternal exposure to polycyclic aromatic hydrocarbons diminishes murine ovarian reserve via induction of Harakiri. J Clin Invest. 2007;117 (12):3971–8.
40. Dechanet C, Anahory T, Mathieu Daude JC, Quantin X, Reyftmann L, Hamamah S, Hedon B, Dechaud H. Effects of cigarette smoking on reproduction. Hum Reprod Update. 2011;17 (1):76–95.
41. Davila JC, Cezar GG, Thiede M, Strom S, Miki T, Trosko J. Use and application of stem cells in toxicology. Toxicol Sci. 2004;79(2):214–23.
42. Brannen KC, Chapin RE, Jacobs AC, Green ML. Alternative models of developmental and reproductive toxicity in pharmaceutical risk assessment and the 3Rs. ILAR J. 2016;57 (2):144–56.
43. Zenzes MT, Puy LA, Bielecki R, Reed TE. Detection of benzo[a]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. Mol Hum Reprod. 1999;5(2):125–31.
44. Poppe WA, Peeters R, Daenens P, Ide PS, Van Assche FA. Tobacco smoking and the uterine cervix: cotinine in blood, urine and cervical fluid. Gynecol Obstet Investig. 1995;39(2):110–4.
45. Fabro S, Sieber SM. Caffeine and nicotine penetrate the pre-implantation blastocyst. Nature. 1969;223(5204):410–1.
46. Thompson J, Bannigan J. Cadmium: toxic effects on the reproductive system and the embryo. Reprod Toxicol. 2008;25(3):304–15.
47. Shiloh H, Lahav-Baratz S, Koifman M, Ishai D, Bidder D, Weiner-Meganzi Z, Dirnfeld M. The impact of cigarette smoking on zona pellucida thickness of oocytes and embryos prior to transfer into the uterine cavity. Hum Reprod. 2004;19(1):157–9.
48. Neal MS, Hughes EG, Holloway AC, Foster WG. Sidestream smoking is equally as damaging as mainstream smoking on IVF outcomes. Hum Reprod. 2005;20(9):2531–5.
49. Hassa H, Gurer F, Tanir HM, Kaya M, Gunduz NB, Sariboyaci AE, Bal C. Effect of cigarette smoke and alpha-tocopherol (vitamin E) on fertilization, cleavage, and embryo development rates in mice: an experimental in vitro fertilization mice model study. Eur J Obstet Gynecol Reprod Biol. 2007;135(2):177–82.
50. Zenzes MT. Smoking and reproduction: gene damage to human gametes and embryos. Hum Reprod Update. 2000;6(2):122–31.
51. Yu HS, Tam PP, Chan ST. Effects of cadmium on preimplantation mouse embryos in vitro with special reference to their implantation capacity and subsequent development. Teratology. 1985;32(3):347–53.
52. Sadeu JC, Foster WG. The cigarette smoke constituent benzo[a]pyrene disrupts metabolic enzyme, and apoptosis pathway member gene expression in ovarian follicles. Reprod Toxicol. 2013;40:52–9.
53. Neal MS, Zhu J, Foster WG. Quantification of benzo[a]pyrene and other PAHs in the serum and follicular fluid of smokers versus non-smokers. Reprod Toxicol. 2008;25(1):100–6.
54. Zhang L, Connor EE, Chegini N, Shiverick KT. Modulation by benzo[a]pyrene of epidermal growth factor receptors, cell proliferation, and secretion of human chorionic gonadotropin in human placental cell lines. Biochem Pharmacol. 1995;50(8):1171–80.
55. Nandi M, Slone D, Jick H, Shapiro S, Lewis GP. Cadmium content of cigarettes. Lancet. 1969;2 (7634):1329–30.
56. Zenzes MT, Krishnan S, Krishnan B, Zhang H, Casper RF. Cadmium accumulation in follicular fluid of women in in vitro fertilization-embryo transfer is higher in smokers. Fertil Steril. 1995;64(3):599–603.
57. Telisman S, Jurasovic J, Pizent A, Cvitkovic P. Cadmium in the blood and seminal fluid of nonoccupationally exposed adult male subjects with regard to smoking habits. Int Arch Occup Environ Health. 1997;70(4):243–8.

58. Chia SE, Xu B, Ong CN, Tsakok FM, Lee ST. Effect of cadmium and cigarette smoking on human semen quality. Int J Fertil Menopausal Stud. 1994;39(5):292–8.
59. Abraham R, Ringwood N, Mankes R. Ultrastructural observations on rabbit blastocysts after maternal exposure to cadmium chloride. J Reprod Fertil. 1984;70(1):323–5.
60. Kawanishi S, Oikawa S. Mechanism of telomere shortening by oxidative stress. Ann N Y Acad Sci. 2004;1019:278–84.
61. Huang Y, Liang P, Liu D, Huang J, Songyang Z. Telomere regulation in pluripotent stem cells. Protein Cell. 2014;5(3):194–202.
62. Liu L, Bailey SM, Okuka M, Munoz P, Li C, Zhou L, Wu C, Czerwiec E, Sandler L, Seyfang A, Blasco MA, Keefe DL. Telomere lengthening early in development. Nat Cell Biol. 2007;9 (12):1436–41.
63. Lodovici M, Akpan V, Evangelisti C, Dolara P. Sidestream tobacco smoke as the main predictor of exposure to polycyclic aromatic hydrocarbons. J Appl Toxicol. 2004;24(4):277–81.
64. Khorram O, Han G, Magee T. Cigarette smoke inhibits endometrial epithelial cell proliferation through a nitric oxide-mediated pathway. Fertil Steril. 2010;93(1):257–63.
65. Garry S, Nesslany F, Aliouat E, Haguenoer JM, Marzin D. Hematite (Fe2O3) acts by oxydative stress and potentiates benzo[a]pyrene genotoxicity. Mutat Res. 2004;563(2):117–29.
66. Pan LQ, Ren J, Liu J. Responses of antioxidant systems and LPO level to benzo(a)pyrene and benzo(k)fluoranthene in the haemolymph of the scallop Chlamys ferrari. Environ Pollut. 2006;141(3):443–51.
67. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408 (6809):239–47.
68. Stampfer MR, Bartholomew JC, Smith HS, Bartley JC. Metabolism of benzo[a]pyrene by human mammary epithelial cells: toxicity and DNA adduct formation. Proc Natl Acad Sci U S A. 1981;78(10):6251–5.
69. Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet. 2000;24(4):372–6.
70. Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, Maruyama M, Maeda M, Yamanaka S. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell. 2003;113(5):631–42.
71. Silva J, Nichols J, Theunissen TW, Guo G, van Oosten AL, Barrandon O, Wray J, Yamanaka S, Chambers I, Smith A. Nanog is the gateway to the pluripotent ground state. Cell. 2009;138 (4):722–37.
72. Sagredo C, Ovrebo S, Haugen A, Fujii-Kuriyama Y, Baera R, Botnen IV, Mollerup S. Quantitative analysis of benzo[a]pyrene biotransformation and adduct formation in Ahr knockout mice. Toxicol Lett. 2006;167(3):173–82.
73. Tscheudschilsuren G, Kuchenhoff A, Klonisch T, Tetens F, Fischer B. Induction of arylhydrocarbon receptor expression in embryoblast cells of rabbit preimplantation blastocysts upon degeneration of Rauber's polar trophoblast. Toxicol Appl Pharmacol. 1999;157 (2):125–33.
74. Matikainen T, Perez GI, Jurisicova A, Pru JK, Schlezinger JJ, Ryu HY, Laine J, Sakai T, Korsmeyer SJ, Casper RF, Sherr DH, Tilly JL. Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nat Genet. 2001;28(4):355–60.
75. Matikainen TM, Moriyama T, Morita Y, Perez GI, Korsmeyer SJ, Sherr DH, Tilly JL. Ligand activation of the aromatic hydrocarbon receptor transcription factor drives Bax-dependent apoptosis in developing fetal ovarian germ cells. Endocrinology. 2002;143(2):615–20.
76. Leng L, Sun J, Huang J, Gong F, Yang L, Zhang S, Yuan X, Fang F, Xu X, Luo Y, Bolund L, Peters BA, Lu G, et al. Single-cell transcriptome analysis of uniparental embryos reveals parent-of-origin effects on human preimplantation development. Cell Stem Cell. 2019;25(5):697–712.
77. Wen L, Tang F. Human germline cell development: from the perspective of single-cell sequencing. Mol Cell. 2019;76(2):320–8.

78. Kucab JE, Zou X, Morganella S, Joel M, Nanda AS, Nagy E, Gomez C, Degasperi A, Harris R, Jackson SP, Arlt VM, Phillips DH, Nik-Zainal S. A compendium of mutational signatures of environmental agents. Cell. 2019;177(4):821–36.
79. Liu J, Liu M, Ye X, Liu K, Huang J, Wang L, Ji G, Liu N, Tang X, Baltz JM, Keefe DL, Liu L. Delay in oocyte aging in mice by the antioxidant N-acetyl-L-cysteine (NAC). Hum Reprod. 2012;27(5):1411–20.
80. Liu M, Yin Y, Ye X, Zeng M, Zhao Q, Keefe DL, Liu L. Resveratrol protects against age-associated infertility in mice. Hum Reprod. 2013;28(3):707–17.
81. Zhang M, Lu Y, Chen Y, Zhang Y, Xiong B. Insufficiency of melatonin in follicular fluid is a reversible cause for advanced maternal age-related aneuploidy in oocytes. Redox Biol. 2019;28:101327.

Chapter 7
Toxicological Effects of BPDE on Dysfunctions of Female Trophoblast Cells

Rong Wang, Xinying Huang, Chenglong Ma, and Huidong Zhang

Abstract Polycyclic aromatic hydrocarbons (PAHs) are widely spread persistent environmental toxicants. Its typical representative benzo[a]pyrene (BaP) is a human carcinogen. BaP can pass through the placental barrier and is finally metabolized into benzo[a]pyren-7, 8-dihydrodiol-9, 10-epoxide (BPDE). BPDE can form DNA adducts, which directly affect the female reproductive health. Based on the special physiological functions of trophoblast cells and its important effect on normal pregnancy, this chapter describes the toxicity and molecular mechanism of BPDE-induced dysfunctions of trophoblast cells. By affecting the invasion, migration, apoptosis, proliferation, inflammation, and hormone secretion of trophoblast cells, BPDE causes diseases such as choriocarcinoma, intrauterine growth restriction, eclampsia, and abortion. In the end, it is expected to provide a scientific basis and prevention approach for women's reproductive health and decision-making basis for the formulation of environmental health standards.

Keywords BaP/BPDE · Trophoblast cell · Invasion and migration · Apoptosis · Cell cycle · Inflammatory cytokines · Hormone secretion

7.1 Basic Introduction of BPDE

Polycyclic aromatic hydrocarbons (PAHs) are mainly produced by fossil fuel and organic materials during incomplete combustion of power generation, industries, residential heating, motor vehicles, etc. In addition, PAHs are also present in tobacco smoke and grills. Exposure to PAHs increases the difficulty of conception and the chance of miscarriage and abnormalities in offspring [1]. Some studies have shown that prenatal PAHs exposure is associated with reduced cognitive function in

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offspring. For example, children in New York City exposed to high levels of PAH in utero had significantly lower Bailey mental development index (MDI) scores, developmentally delayed at the age of 3 [2], and an increased probability of low IQ scores at 5 years of age [3]. The results of a prospective cohort study in Poland suggest that prenatal exposure to airborne PAHs is detrimental to cognitive development in children by age 5 [4].

Benzo[a]pyrene (BaP) is a representative of PAHs and is listed as a human carcinogen by the International Agency for Research on Cancer [5]. In industrial or domestic activities, such as cooking with oil or wood burning, ambient air concentrations of BaP range from 20 mg/m3 to 100 mg/m^3 [6]. High concentrations of BaP are found in smoked beef dried, fried chicken, and some potato chips, based on daily food intake and levels of environmental BaP contaminants, the total intake of BaP was estimated to be 125 ng/person/day [7]. BaP has a long half-life and is lipophilic, leading to a high level accumulation in the body. For smoking women who smoke 12–24 cigarettes a day, the concentration of BaP in the serum was twice higher than that of non-smokers, and BaP is 4–10 ng/mL in follicular fluid. The total concentration of BaP and its metabolites range from 40 to 100 ng/mL [8].

BaP can be absorbed by oral, inhalation, and cutaneous contact of exposure. After absorption by human, BaP is metabolically activated by cytochrome P4501A1 and epoxide hydrolase induced by aromatic hydrocarbon receptors, forming the carcinogenic Benzo(a)pyrene 7, 8-dihydrodialcohol-9, 10-epoxide (BPDE). Many studies have demonstrated that BaP can cross the placental barrier, leading to placental and fetal developmental toxicity [9]. BaP and its metabolites can be transported from the mother to the fetal compartment by human placental perfusion model [10]. Pregnant women who were exposed to PAHs had increased levels of BaP and its metabolites in urine, placenta, cord blood, maternal blood, and breast milk and increased levels of BPDE-DNA adducts in maternal and neonatal white blood cells [11–13].

BaP is an endocrine-disrupting chemical, exposure to BaP has adverse effects on endocrine, reproductive, immune, and nervous systems. It reduces serum levels of progesterone, 17β-estradiol, prolactin, and other pregnancy-related hormones, interferes with embryonic development, and reduces fetal survival [14]. Moreover, BaP affects learning and memory by affecting the hippocampus and cortical neural membrane, and it also induces tumor onset [5]. BPDE can form covalent BPDE-DNA adducts through guanine N^2 and cause DNA damage, mutation, and carcinogenesis. High level of BPDE-DNA adducts in aborted tissues and maternal blood may increase the risk of abortion during early pregnancy [15].

BPDE-DNA adducts can also adversely affect the development of blastocysts and significantly reduce the success rate of pregnancy [16]. In addition, high levels of BPDE-DNA were associated with lower birth weight, birth length, and head circumference for the newborns. An earlier study in New Jersey, USA, showed that women with higher prenatal PAHs exposure had significantly increased risks of death, preterm birth, and low birth weight in their offspring [17]. A case-control study conducted in Tianjin, China, showed that the level of BPD-DNA adducts in the blood in the experienced missed abortion group was significantly higher than that

in the control group. Animal experiments also demonstrated that prenatal exposure to BaP significantly reduced fetal survival and birth weight in a dose-dependent manner [14]. In summary, BaP can cross the placental barrier and be transferred to the fetus, causing trophoblast cell dysfunction, fetal and placental developmental toxicity.

7.2 Function of Trophoblast Cells and their Relation to Disease

Implantation plays an important role in the successful pregnancy, the process of implantation into the uterus includes adhesion, migration, and invasion. Cytotrophoblast cells are primitive placental cells produced by the blastocyst trophoblast ectoderm and divide into two main cell populations. One is villous trophoblast cells, by cell proliferation and fusion, which generate syncytiotrophoblast neighboring the maternal blood sinus, forming villous branches covered by epithelial cells. This is the unit of nutrition and gas exchange between the mother and the embryo, which ensures the basic nutritional exchange and endocrine function. Successful pregnancy requires effective proliferation and differentiation of villous trophoblast cells. The other is extravillous trophoblast, which is the root of the chorionic villi. Extravillous trophoblasts cells migrate and invade the uterine decidual, decidual artery, and uterine spiral artery, helping the placenta attached to the endometrium. Properly and strictly control of extravillous trophoblastic cell migration and invasion is necessary for the healthy growth of the fetus in the maternal uterus.

Trophoblast invasion has many similarities with tumor invasion. However, unlike uncontrolled tumor invasion, successful implantation of an embryo requires two physiological invasions to the endometrium, at the beginning of pregnancy and at 14–16 weeks of gestation, the invasion depth is upper third of the myometrium. Trophoblast invasion is strictly limited in space and time [18].

Compared with the endometrium, embryonic cells have higher proliferation rate, physiological immaturity, lower detoxification ability, and lower immune response ability; thus, the embryo is more sensitive to environmental chemicals [19, 20]. Inadequate invasion and migration of extracorporeal trophoblast cells are associated with uterine spiral artery remodeling disorder, leading to pregnancy failure, intrauterine growth restriction [21], preeclampsia (PE), eclampsia [22], or abortion [23]. However, excessive invasion of EVTs results in invasive hydatidiform mole and choriocarcinoma. In all human pregnancies in an American Indian population, 2–8% are classified as PE and eclampsia, which cause approximately 50,000 maternal deaths annually [24–26]. Therefore, moderate EVTs invasion plays an important role in mammalian placental development and human pregnancy success. Unhealthy trophoblasts are always correlated with inflammation [27], oxidative stress [28], apoptosis [29], and a dysregulated angiogenic profile [30].

7.3 BPDE Results in Dysfunctions of Trophoblast Cells

7.3.1 BPDE Inhibits the Invasion and Migration

The placenta is essential for a healthy pregnancy because it supports the growth of the baby, helps the mother's body adaption, and provides a connection between mother and the developing baby. BaP can cross the placental barrier and transfer from the mother to the embryo, causing fetal and placental developmental toxicity.

Animal studies have shown that exposure to BaP in mice can damage endometrial receptance, reduce the number of implantation sites, and impair decidualization and decidual angiogenesis in early pregnancy [31]. Trophoblast cell invasion is precisely regulated by many signaling pathways, such as TGF-β-dependent Smad factors, PI3K-Akt, FAK, SRC, and MAKP signal pathways.

Studies have confirmed that BaP alters the migration and invasion of extravillous trophoblast. Using EVTs cells HTR8/SVneo as the experimental model, BaP exposure inhibited the migration and invasion of trophoblast cells by activating ERK and JNK signal pathways [32]. BPDE (a carcinogenic metabolite of BaP) exposure inhibited the migration and invasion of HTR-8/SVneo cells by inhibiting the FAK/SRC/PI3K/AKT pathway [33]. Furthermore, similar results are obtained at extracellular trophoblast Swan 71, BPDE inhibited the PI3K/AKT/CDC42/PAK1 signaling pathway by upregulating Mir-194-3p and finally inhibited the filopodia formation and migration/invasion of Swan71 cells [34].

7.3.2 BPDE Promotes Trophoblast Cell Apoptosis

Apoptosis is cell suicide activity under the control of genetic coding procedures during the development of an individual cell. Apoptosis is the process of the body's active response to external stimuli, which can be seen in embryo development, normal tissue metabolism, and certain pathological conditions.

The placental trophoblast is the tissue that exchanges oxygen, nutrients, and metabolites between the mother and the fetus. A large number of studies have confirmed that trophoblast apoptosis is a physiological phenomenon and has important physiological significance [35, 36]. During the development of the placenta, moderate apoptosis is conducive to the formation of the placental vascular lumen and branches [37]. It is generally believed that the imbalanced regulation of apoptosis which leads to accelerated apoptosis is the root cause of many female diseases. More and more researchers are concerned about the relationship between trophoblast cell apoptosis and pregnancy-related diseases. Halperin et al. have reported in 2000 that excessive apoptosis of placental trophoblast cells was increased in patients with ectopic pregnancy [38].

Exposure to PAHs during women pregnancy leads to imbalanced regulation of apoptosis. Treatment of extravillous trophoblast HTR-8/SVneo cells with BaP or

BPDE significantly reduces cell survival rate and induces different degrees of cell apoptosis [39]. However, the molecular mechanism of PAHs-induced apoptosis is limited. One study confirmed that after human trophoblast cell Swan 71 was exposed to BPDE, pro-apoptosis proteins P53 and Bak1 were increased, and anti-apoptosis protein Bcl-2 was decreased. Furthermore, mitochondrial fusion genes (Mfn1, Mfn2, and OPA1) were decreased, and those of fission genes (Fis1 and Drp1) were increased, resulting in the release of cytochrome C and activation of Caspase 3, which irreversibly induced trophoblast cell apoptosis [40].

7.3.3 BPDE Affects Cell Cycle Function

Cell cycle refers to the whole process that a cell undergoes from the completion of one division to the end of the next division, which is divided into two stages: the interphase and the mitosis phase. The process of the growth of new cells produced by cell mitosis until the end of the next cell mitosis to form daughter cells is generally called the cell cycle. Life is a continuous process of passing from one generation to the next. It is a process of constantly updating and starting from scratch. Therefore, the normal functions of the cell cycle are vital to life. And successful embryo implantation requires a normal growth environment to ensure normal cell mitosis.

BPDE can covalently react with extracyclic deoxyguanosine (90%) and deoxyadenosine (10%) residues in genomic DNA, thereby generating large DNA adducts. Due to the potential threat of DNA adducts and other forms of DNA damage to genome stability, cells have developed elaborate mechanisms to identify and repair damaged DNA. Cell cycle checkpoints are signal transduction pathways that can react with damaged DNA by inhibiting cell cycle progression. BaP can cause a wide range of cell cycle disturbances, including G0/G1 blockage, G2/M blockage, S-phase accumulation, reducing DNA replication capacity and inducing cell proliferation inhibition [8]. One study has confirmed that cell cycle was arrested at G0/G1 phase after exposure of extravillous trophoblast HTR-8/SVneo to BPDE [33]. Because the cell cycle is blocked, cells have adequate time to repair DNA damage.

7.3.4 BPDE Promotes the Expression of Inflammatory Cytokines

Successful pregnancy requires implantation and invasion of trophoblast. The invasive process occurs in the initial pregnancy, no more than one-third of the myometrium. The process of implantation and trophoblast infiltration requires an inflammatory environment. Successful implantation requires an inflammatory environment, which is achieved by the proper education of the innate immune cells that

invade the trophoblast [41], in which the interaction of trophoblast monocytes may be the key to the factors necessary for full-term pregnancy. According to the levels of cytokines and chemokines in pregnant women's serum, pregnancy can consist of three stages. Early pregnancy is linked to increased production of proinflammatory cytokines and chemokines, such as IL-8 and MCP1, and their presence in maternal circulation. An anti-inflammatory phase was observed in the second trimester and proinflammatory cytokines were produced in the third trimester, which is considered essential for delivery [42]. Under normal conditions, trophoblast derived exosomes can recruit and "educate" monocytes to produce proinflammatory cytokines/chemokines in a manner unrelated to cell contact [43].

During the invasion of trophoblast cells into endometrium, more mitochondria are needed in order to provide energy for hormone synthesis and trophoblast oxygen sensing [44] and trophoblast oxygen sensing [45]. Mitochondria are sensitive organelles of oxidative stress and environmental toxicants. Mitochondrial dysfunction leads to excessive ROS production. If excessive ROS cannot be removed by antioxidants in time, ROS will lead to cell damage through lipid peroxidation [46, 47]. The production of oxidative stress, lipid peroxidation, and lack of antioxidant defense are related to trophoblast related diseases, such as preeclampsia [48], growth restriction [49], and abortion [50]. Urrutia et al. confirm that there is a close relationship between mitochondrial dysfunction and severe inflammatory response. This combination increased levels of proinflammatory factors TNF-a and IL-6 [51].

Studies have confirmed that BaP activated the primordial follicle, formed follicular atresia in vitro and in vivo, increased mitochondrial reactive oxygen species (ROS) and membrane lipid peroxidation, damaged the fluidity of the egg membrane, and reduced fertilization in adult mice [52]. In addition, with increasing BPDE concentration, TNF-α and IL-6 mRNA levels in Swan 71 cells gradually increase, and the increased expression of proinflammatory factors resulted in the inflammatory response. BPDE promotes this expression of inflammatory factors by inducing oxidative damage of mitochondria. After exposure to BPDE, the protein expression levels of mitochondrial fusion genes mfn1, Mfn2, and OPA1 decrease, and the protein expression levels of mitotic genes FIS1 and Drp1 increase, resulting in the release of Cyt C and the activation of caspase 3 [40]. These changes cause oxidative stress in trophoblast cells, activate NF-κB pathway, release proinflammatory factors, and aggravate inflammatory response and oxidative damage [53].

7.3.5 BPDE Inhibits Trophoblast Cell Hormone Secretion

Human chorionic gonadotropin (hCG) is an essential hormone for the establishment, promotion, and maintenance of human pregnancy [54]. In early pregnancy, hCG is mainly generated by differentiated syncytiotrophoblast, which represents a key embryonic signal [55], which is essential for maintaining pregnancy. HCG is a variety of endocrine, paracrine, and autocrine roles in various pregnant and nonpregnant cells and tissues. These effects are designed to promote trophoblast

invasion and differentiation, placental growth, angiogenesis of uterine blood vessels, hormone production, to regulate the maternal-fetal interface immune system, and to inhibit myometrial contractility and fetal growth and differentiation [54]. In the first 6 weeks of pregnancy, hCG promotes the secretion of progesterone, estradiol, and estrone by transforming the ovaries after ovulation into the corpus lutein of pregnancy [56].

Some studies reported that endocrine disruption (EDCs) can affect the reproductive system of various [57]. BPDE has been proven to be an endocrine-disrupting chemical, which can interfere with the reproductive process, reduce the levels of progesterone, 17 β-estradiol, and prolactin pregnancy-related hormones in serum, and change the fetal survival rate [14]. Compared with the control group, the pregnancy rate of PAHs-treated mice decreases by about 40%, and the number of germ cells reduces by 20% [58]. After pregnant rats were exposed to 25, 75, or 100 mg/m^3 BaP, the concentrations of progesterone, estrogen, and prolactin in the plasma decrease gradually, and the fetal survival rate also decreases in a dose-dependent manner [14]. Trophoblast cells are the main hormone secreting cells and reduce the corresponding hormone secretion when their functions are damaged. EVT Swan 71 cells also secrete hCG, which promotes trophoblast invasion and maintains progesterone in early pregnancy. After treatment of swan-71 cells with different concentrations of BPDE for 24 h, with the increase of BPDE concentration, the expression level of hCG and hCG β protein in swan-71 cells decreased gradually.

In summary, BPDE can inhibit the invasion and migration, promote apoptosis, affect the cycle of trophoblast, and strengthen the expression of inflammatory factors with trophoblast, leading to dysfunctions of trophoblast and the decrease of hormone secretion.

References

1. Lee B-E, et al. Secondhand smoke exposure during pregnancy and infantile neurodevelopment. Environ Res. 2011;111(4):539–44.
2. Perera FP, et al. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. Environ Health Perspect. 2006;114(8):1287–92.
3. Perera FP, et al. Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5 years. Pediatrics. 2009;124(2):e195–202.
4. Edwards SC, et al. Prenatal exposure to airborne polycyclic aromatic hydrocarbons and children's intelligence at 5 years of age in a prospective cohort study in Poland. Environ Health Perspect. 2010;118(9):1326–31.
5. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum. 2010;92:1–853.
6. Wormley DD, Ramesh A, Hood DB. Environmental contaminant-mixture effects on CNS development, plasticity, and behavior. Toxicol Appl Pharmacol. 2004;197(1):49–65.
7. Lee BM, Shim GA. Dietary exposure estimation of benzo[a]pyrene and cancer risk assessment. J Toxicol Environ Health A. 2007;70(15–16):1391–4.

8. Xie Y, et al. Benzo(a)pyrene causes PRKAA1/2-dependent ID2 loss in trophoblast stem cells. Mol Reprod Dev. 2010;77(6):533–9.
9. Arnould JP, et al. Detection of benzo[a]pyrene-DNA adducts in human placenta and umbilical cord blood. Hum Exp Toxicol. 1997;16(12):716–21.
10. Mathiesen L, et al. Transport of benzo[alpha]pyrene in the dually perfused human placenta perfusion model: effect of albumin in the perfusion medium. Basic Clin Pharmacol Toxicol. 2009;105(3):181–7.
11. Neal MS, Zhu J, Foster WG. Quantification of benzo[a]pyrene and other PAHs in the serum and follicular fluid of smokers versus non-smokers. Reprod Toxicol. 2008;25(1):100–6.
12. Madhavan ND, Naidu KA. Polycyclic aromatic hydrocarbons in placenta, maternal blood, umbilical cord blood and milk of Indian women. Hum Exp Toxicol. 1995;14(6):503–6.
13. Ptashekas J, et al. Environmental and health monitoring in Lithuanian cities: exposure to heavy metals and benz(a)pyrene in Vilnius and Siauliai residents. J Environ Pathol Toxicol Oncol. 1996;15(2–4):135–41.
14. Archibong AE, et al. Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo(a)pyrene. Reprod Toxicol. 2002;16(6):801–8.
15. Wu J, et al. Exposure to polycyclic aromatic hydrocarbons and missed abortion in early pregnancy in a Chinese population. Sci Total Environ. 2010;408(11):2312–8.
16. Iannaccone PM, Fahl WE, Stols L. Reproductive toxicity associated with endometrial cell mediated metabolism of benzo[a]pyrene: a combined in vitro, in vivo approach. Carcinogenesis. 1984;5(11):1437–42.
17. Vassilev ZP, Robson MG, Klotz JB. Associations of polycyclic organic matter in outdoor air with decreased birth weight: a pilot cross-sectional analysis. J Toxicol Environ Health A. 2001;64(8):595–605.
18. Burrows TD, King A, Loke YW. Trophoblast migration during human placental implantation. Hum Reprod Update. 1996;2(4):307–21.
19. Makri A, et al. Children's susceptibility to chemicals: a review by developmental stage. J Toxicol Environ Health B Crit Rev. 2004;7(6):417–35.
20. Barr DB, Bishop A, Needham LL. Concentrations of xenobiotic chemicals in the maternal-fetal unit. Reprod Toxicol. 2007;23(3):260–6.
21. Weiss G, et al. The trophoblast plug during early pregnancy: a deeper insight. Histochem Cell Biol. 2016;146(6):749–56.
22. Kadyrov M, et al. Pre-eclampsia and maternal anaemia display reduced apoptosis and opposite invasive phenotypes of extravillous trophoblast. Placenta. 2003;24(5):540–8.
23. Ball E, et al. Late sporadic miscarriage is associated with abnormalities in spiral artery transformation and trophoblast invasion. J Pathol. 2006;208(4):535–42.
24. Duley L. Pre-eclampsia and the hypertensive disorders of pregnancy. Br Med Bull. 2003;67:161–76.
25. Karumanchi SA, Granger JP. Preeclampsia and pregnancy-related hypertensive disorders. Hypertension. 2016;67(2):238–42.
26. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308 (5728):1592–4.
27. Cindrova-Davies T, et al. Nuclear factor-kappa B, p38, and stress-activated protein kinase mitogen-activated protein kinase signaling pathways regulate proinflammatory cytokines and apoptosis in human placental explants in response to oxidative stress: effects of antioxidant vitamins. Am J Pathol. 2007;170(5):1511–20.
28. Leach RE, et al. Diminished survival of human cytotrophoblast cells exposed to hypoxia/reoxygenation injury and associated reduction of heparin-binding epidermal growth factor-like growth factor. Am J Obstet Gynecol. 2008;198(4):471.e1–7; discussion 471.e7–8.
29. Huppertz B. Trophoblast differentiation, fetal growth restriction and preeclampsia. Pregnancy Hypertens. 2011;1(1):79–86.
30. Sgambati E, et al. VEGF expression in the placenta from pregnancies complicated by hypertensive disorders. BJOG. 2004;111(6):564–70.

31. Li X, et al. Exposure to benzo[a]pyrene impairs decidualization and decidual angiogenesis in mice during early pregnancy. Environ Pollut. 2017;222:523–31.
32. Liu L, et al. Benzo(a)pyrene inhibits migration and invasion of extravillous trophoblast HTR-8/SVneo cells via activation of the ERK and JNK pathway. J Appl Toxicol. 2016;36(7):946–55.
33. Wang R, et al. Benzo[a]pyrene-7,8-diol-9,10-epoxide suppresses the migration and invasion of human extravillous trophoblast HTR-8/SVneo cells by down-regulating MMP2 through inhibition of FAK/SRC/PI3K/AKT pathway. Toxicology. 2017;386:72–83.
34. Tian Z, et al. Benzo[a]pyrene-7, 8-diol-9, 10-epoxide suppresses the migration and invasion of human extravillous trophoblast swan 71 cells due to the inhibited filopodia formation and down-regulated PI3K/AKT/CDC42/PAK1 pathway mediated by the increased miR-194-3p. Toxicol Sci. 2018;166(1):25–38.
35. Smith SC, Baker PN, Symonds EM. Placental apoptosis in normal human pregnancy. Am J Obstet Gynecol. 1997;177(1):57–65.
36. Nelson DM. Apoptotic changes occur in syncytiotrophoblast of human placental villi where fibrin type fibrinoid is deposited at discontinuities in the villous trophoblast. Placenta. 1996;17(7):387–91.
37. Tertemiz F, et al. Apoptosis contributes to vascular lumen formation and vascular branching in human placental vasculogenesis. Biol Reprod. 2005;72(3):727–35.
38. Halperin R, et al. Placental apoptosis in normal and abnormal pregnancies. Gynecol Obstet Investig. 2000;50(2):84–7.
39. Maul RW, Sutton MD. Roles of the Escherichia coli RecA protein and the global SOS response in effecting DNA polymerase selection in vivo. J Bacteriol. 2005;187(22):7607–18.
40. Wang W, et al. Benzo(a)pyren-7,8-dihydrodiol-9,10-epoxide induces human trophoblast Swan 71 cell dysfunctions due to cell apoptosis through disorder of mitochondrial fission/fusion. Environ Pollut. 2018;233:820–32.
41. Mor G, Koga K. Macrophages and pregnancy. Reprod Sci. 2008;15(5):435–6.
42. Fest S, et al. Trophoblast-macrophage interactions: a regulatory network for the protection of pregnancy. Am J Reprod Immunol. 2007;57(1):55–66.
43. Atay S, et al. Trophoblast-derived exosomes mediate monocyte recruitment and differentiation. Am J Reprod Immunol. 2011;65(1):65–77.
44. Tuckey RC. Progesterone synthesis by the human placenta. Placenta. 2005;26(4):273–81.
45. De Marco CS, Caniggia I. Mechanisms of oxygen sensing in human trophoblast cells. Placenta. 2002;23(Suppl A):S58–68.
46. Afanas'ev IB. Signaling functions of free radicals superoxide & nitric oxide under physiological & pathological conditions. Mol Biotechnol. 2007;37(1):2–4.
47. Valko M, et al. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44–84.
48. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med. 1999;222(3):222–35.
49. Myatt L. Review: reactive oxygen and nitrogen species and functional adaptation of the placenta. Placenta. 2010;31(Suppl):S66–9.
50. Poston L, Raijmakers MT. Trophoblast oxidative stress, antioxidants and pregnancy outcome--a review. Placenta. 2004;25(Suppl A):S72–8.
51. Jaffe EA, et al. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J Clin Invest. 1973;52(11):2745–56.
52. Sobinoff AP, et al. Jumping the gun: smoking constituent BaP causes premature primordial follicle activation and impairs oocyte fusibility through oxidative stress. Toxicol Appl Pharmacol. 2012;260(1):70–80.
53. Lenaers G, et al. OPA1 functions in mitochondria and dysfunctions in optic nerve. Int J Biochem Cell Biol. 2009;41(10):1866–74.
54. Paulesu L, et al. hCG and its disruption by environmental contaminants during human pregnancy. Int J Mol Sci. 2018;19(3):914.

55. Fournier T, Guibourdenche J, Evain-Brion D. Review: hCGs: different sources of production, different glycoforms and functions. Placenta. 2015;36(Suppl 1):S60–5.
56. Hay DL. Placental histology and the production of human choriogonadotrophin and its subunits in pregnancy. Br J Obstet Gynaecol. 1988;95(12):1268–75.
57. Takamiya M, Lambard S, Huhtaniemi IT. Effect of bisphenol A on human chorionic gonadotrophin-stimulated gene expression of cultured mouse Leydig tumour cells. Reprod Toxicol. 2007;24(2):265–75.
58. Detmar J, et al. Embryonic loss due to exposure to polycyclic aromatic hydrocarbons is mediated by Bax. Apoptosis. 2006;11(8):1413–25.

Chapter 8
The Roles of Stress-Induced Immune Response in Female Reproduction

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Abstract Stress response plays pivotal roles in physiological process, including reproduction and embryonic development. It's long been acknowledged that stress stimulates the activation of both hormone and immune system resulting in disorders of maternal immune function and infertility. However, the stress types, biological alterations, clinical outcomes, and the potential underlying mechanisms remain largely unclear. Recent studies suggest that more stress factors and relative mechanisms are identified to be involved in female reproductive immune response stimulation, and they may lead to immune dysregulations that negatively influence maternal health. In this part, we focus on the outcomes or mechanisms of common stress factors which affect female immune response before and during pregnancy.

Keywords Stress · Immune response · Reproductive disorders · Signaling pathways

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8.1 Introduction

Maternal stress exposure is a nonspecific risk factor causing neurodevelopmental outcomes in subsequent offspring [1]. It will also cause psychopathologies such as autism, schizophrenia, and anxiety problem in human [2–4], and lead to an increased anxiety, a decreased sociability, and deficits in cognitive and motor function in animal models [5, 6]. Increasing evidence suggests that maternal stress exposure will affect offspring development. The psychological maternal condition, intrauterine factors, hormone and immune responses are vital to the healthy development of the embryo. Maternal stress is suggested to play an essential role in the adaptation of maternal dynamic hormones and immune response, and to be capable of negatively affecting the development of the fetus with a long-term influence [7].

Although studies of model animals and epidemiological survey show that maternal stress exposure negatively affects the neurodevelopment of the offspring, few studies find that the relationship between stress exposure and female immune functions is altered. Stress has the capability of altering the maternal inflammatory responses of spleen and lymphocytes, promoting the secretion of inflammatory cytokines [8]. Another theory suggests that interplay(s) between the maternal immune reaction and the fetus brain is a latent mechanism by which maternal stress could affect the neurodevelopment [4]. However, whether maternal stress exposure could also change the fetus immune response and whether have a negative influence on the offspring remain largely unknown.

Current studies suggest that the maldevelopment of fetus caused by maternal stress exposure might be a result of interaction between maternal immune activation and embryo or fetus development adaptation. Evidence shows that obvious activation of specific lymphocytes [9], cytokines [10], and immune reactive pathway is observed in both human and animal models that are under stress. Hence, we will briefly summarize the maternal stress that women might be exposed to before and during pregnancy. And we will also have a look into the clinical outcomes and molecular alterations induced by maternal stress or the signal pathways. These signal pathways mean a consequence of stress-induced immune responses when maternal stress stimulates the downstream signals and influences the mother and her fetus.

8.2 Stress Exposure

During pregnancy, pregnant woman and her fetus might be confronted with various stresses which might result in significant changes of the mother's physiological conditions and a maldevelopment of the fetus's nerve and immune system. Such stresses can be divided into several groups according to the time they may occur.

8.2.1 Pregestational Stress

Pregestational stress refers to chronic stress which is suffered by a woman prior to gestation. It has been shown that pregestational stress can negatively affect offspring's immune, cardiovascular system and neurobehavioral development [11, 12]. Pregestational chronic maternal stress can also lead to a significant decrease in fertility rate as indicated in the study of dams [13]. However, to date, no study has directly examined the relationship between pregestational maternal stress and immune alterations, and the link of pregestational maternal stress and changes of offspring immune functions.

8.2.2 Prenatal Stress

Prenatal stress is also known as prenatal maternal stress that occurs during pregnancy, which is closely related to developmental plasticity and it is considered a risk and dangerous factor for the development of the offspring [14]. However, a long-term observation suggests that stress from pregnancy seriously affects offspring's development. Previous studies indicate that antenatal stress is associated with greater behavior and physiological reaction in early stage of life, increasing offspring' reaction to both sustaining and non-sustaining rearing experiences [15, 16]. Animal study also shows that prenatal stress affects developmental plasticity by inducing greater environmental sensitivity, becoming a risk factor while facing adversity and a factor while in a supportive environment [17]. Yet, it remains unclear how such environmental sensitivity is induced. Many animal models researches suggest that stress from maternal part before pregnancy may change the environment in utero and take effects on placental construction or function during the period of organ growth and development, causing physiological adaptations in the developing fetus that have ongoing effects on persistent postnatal development [1]. In short, more and more researches indicate that prenatal stress has a negative influence on both mother and offspring.

Prenatal maternal stress exposure in both animal models and humans has related to a wide array of psychiatric and neurodevelopmental disorders and problems in resultant offspring [1]. The clinical manifestations of prenatal stress include higher levels of sadness, aggression, anxiety, and physical disorders [18]. Prenatal maternal stress might negatively influence the development of fetal brain by constricting the placental arteries, resulting in a reduction of fetal blood flow as well as the essential nutrients and oxygen supply [19, 20]. Prenatal stress might also result female embryo become to masculinization [21]. Recent evidence suggests that prenatal maternal stress may increase autism (autism spectrum disorder, ASD) risk or increase the variability in autism-like traits in the large population.

Prenatal stress may also negatively affect the activation of mother's immune system and further influence the development of offspring. Besides, maternal

immune system activation caused by prenatal stress might also contribute to mental disease risk and abnormal offspring behavior [4]. Previous studies have shown that specific cytokines, including interleukin-2 (IL-2), interleukin-6(IL-6), and IL-17A during pregnancy are linked to offspring neuropsychiatric disorders [22–24]. It is notable that IL-6 is likely to be of importance in the etiology of prenatal stress effects [25]. Besides, IL-6 is suggested to be a potential mediator of prenatal maternal immune activation which will affect offspring neurodevelopment [26, 27]. Animal models also show that under prenatal stress, maternal cytokine levels also alter, with a significant increase of circulating IL-6 [28]. IL-6 is essential for increased embryonic multivacuolated microglia and for persistent microglia changes [4]. Stress exposure can also activate the transcription factor NF-κB in mononuclear cells derived peripheral blood, increasing its circulating numbers of pro-inflammatory cytokines [29].

In addition to the alteration of cytokine levels, prenatal stress also affects the lymphocyte population and immune cell functions. Prenatal stress can significantly decrease the serum immunoglobulin G (IgG) concentrations in suckling pigs and has an immune suppressive effect on lymphocyte proliferation because of the T-cell mitogen concanavalin A (ConA) [30]. In addition, prenatal stress can also inhibit the response to B-cell mitogens lipopolysaccharide (LPS) in suckling pigs [30]. Compared to healthy, non-pregnant women, women under diabetes stress during pregnancy are reported to show obvious changes in lymphocyte sub-populations, such as the population of naïve T cells are decreased, while the population of memory T-cells and activated T cells (CD4 + HLA-DR+, CD4 + CD29+) are higher [31].

8.2.3 Perinatal Stress

Perinatal stress is reported to have a gender-dependent effect on offspring [32]. It can also intensify the risk of the development of psychoneurological, immunological, and psychological disorders and decrease the reproduction of the offspring [33]. Animal study also shows that perinatal stress can lead to learning impairment, increased anxiety and depressive behaviors, and enhanced sensitivity to drugs of abuse via fetal programming [32, 34]. Study of adult rats suggests that perinatal stress might cause an elevation of ACTH level in cell type of lymphocytes, monocytes and granulocytes, and mast cells, provoking a life-long hormonal imprinting [35]. It is notable that the inflammasome might be an important immune molecular between stress, neuroendocrine and inflammatory process [36]. In the brain, microglia are seen as the primary immune cells. Microglia and toll-like receptor 4 play a vital role in triggering various stress responses caused by the activation of the inflammasome, which increases the level of inflammatory cytokines, elevated serotonin metabolism, or decreased neurotransmitter availability as well as hypothalamic–pituitary–adrenal (HPA) axis hyperactivity [37]. During pregnancy, intricate neuroimmune communication network would dysregulate always, and it would change the maternal

milieu, enhancing the emergence of depressive symptoms, even negative obstetric as well as neuropsychiatric outcomes [37].

8.2.4 Neonatal Stress

Neonatal stress refers to stress offspring confronted by during neonatal period. Neonatal stress can also have long-term influences on neurotransmitter system and brain, which could increase the risk of vulnerability in later life [38]. A close relationship between neonatal stress and immune function disorder is observed in animal studies. Known study reveals that neonatal stress can augment inflammatory cytokine level and viral replication when adult mice were infected by influenza virus [39, 40]. Animal model study further displays that neonatal stress damages the regulation of innate resistance resulting in immunological and behavioral responses abnormally increased when immune activated, which might even have a long-lasting effect on the susceptibility to diseases [41]. Neonatal stress as premature weaning leads to lymphocyte proliferation suppression and a higher risk of premature deaths in rat and reduced proliferation of lymphocyte in response to B or T cell mitogens in monkeys [40, 42]. Stress on separation of rat pups from their dams could result in significant aggravation in the severity of experimental autoimmune encephalomyelitis (EAE), it would aggravate the severity of airway inflammation in an asthma rat experiment and decrease in serum immunoglobulin levels in mice treated with injection of sheep red blood cells [43–45]. The possible mechanism(s) underlying neonatal stress-mediated negative regulation on immunity is still unclear. Studies using the maternal separation (MSP) experimental model find that the response of the HPA axis to stress is augmented and, at the same time, glucocorticoid feedback control is altered [41, 46, 47]. Hence, it is supposed that the activation of HPA axis might regulate the inflammatory response, activating the immune system accompanied by various behavioral changes [41].

As described above, both the mother and her fetus are influenced by psychosocial and biological stress. Maternal stress exposure in humans has been related to the psychiatric and neurodevelopmental disorders in offspring [1]. While stress was found to be associated with lower immunoglobulin G (Ig) production, reduced immune function, and elevated IL-6 and IL-1β in the first and third trimester, the mechanism through which immune modulation is conducted during pregnancy is still unclear [10, 48]. Generally, maternal immune system remains in multifaceted and dynamic state, being immune-tolerant to fetal cells and protecting maternal cells from pathogen attack at the same time. Besides, it is well acknowledged that the interaction between HPA axis and immune system plays a pivotal role in adaptation when mother is pregnant. Additionally, under stress, HPA axis activation during pregnancy is observed in both human and animal studies. Thus, it is supposed that after stress exposure, alterations in maternal HPA axis result in modulation of immune response. Namely, HPA axis might be an important mediator between prenatal stress and immune functioning [32, 49]. It is also supposed that stress-

relevant neurocircuitry and immunity form an integrated system which is involved in innate and adaptive immune systems interacting with neurotransmitters and neurocircuits to influence the risk for stress [36]. Psychosocial stress causes inflammasome activation and then the stress-induced inflammatory signals are transmitted to the brain, resulting in behavioral responses.

8.3 Stress-Induced Activation of Hormone and Immune System

Stress will break the balance of endocrine and immune system of the body and cause the disorder of the whole internal environment, which is mainly expressed as abnormal hormone secretion level and the activation of the immune system. Under the circumstances of acute or strong stress, the cell activates a series of signaling pathways and then responds to stress state through the expression of related genes. The following is a brief introduction from four aspects, cytokine, hormone secretion and signaling, immune cell activation, and immune regulatory gene expression and modification.

8.3.1 Cytokine

The current study focused on two groups of female who are pregnant or infertile. There is evidence shown that secretion of inflammatory cytokines is increased due to excessive exposure to emergency conditions during pregnancy [10]. In individuals, cytokine levels are not stable during pregnancy, and IL-6 and TNF-α are significantly increased [50]. When pregnant women experience trauma, the levels of TNF-α could be significantly higher than normal pregnant women. For women who repeatedly miscarry or remain infertile, their physiological emotion stages, such as stress, anxiety, and depression, cause abnormal cytokine levels and directly relate to the IVF poor outcomes. Researchers conduct stress scale investigation for patients with infertility and detect cytokines from blood, cervicovaginal fluid, and follicular fluid. The cytokines such as TGF-β in serum are lower, while IL-6 and IL-1β in cervicovaginal fluid are higher than normal pregnant woman [51].

8.3.2 Hormone Secretion and Signaling

It is currently believed that stress causes changes in a variety of female hormones that affect the function of the reproductive system, such as abnormal ovulation, premature ovarian failure (POF), infertility, and so on. The stress-regulated

hormones can be classified into two types, nitrogen hormone and steroid hormone. Nitrogenous hormones include insulin and corticotropin-releasing hormones (CRH). Researches have shown that pregnancy women who have experienced more stress assessed by maternal STAI trait stress score have decreased insulin sensitivity but increased corticotropin-releasing hormones [52]. Abnormal insulin levels can lead to stress hyperglycemia, even increase the risk of diabetes. High levels of CRH through the placenta cause preterm birth and intrauterine developmental retardation (IDR), even cause neonatal neural development disorders and adverse effects on psychological health potentially [53]. Steroid hormone includes cortisol, estrogen, progesterone, and androgens. The ovulation cycle of healthy women without reproductive disease will be changes when exposed to pressure source [54]. The specific mechanism is closely related to the cortisol changes. According to Karen C. Schliep, with increased stress levels, estrogen (E2), progesterone, and luteinizing hormone (LH) are decreased, and women with high stressors are more likely to be anovulatory [54]. Based on a prospective study which explored correlation between the biomarkers of the stress evaluation and pregnancy loss, researchers didn't find clear association between cortisol and adverse pregnancy outcomes [55]. In addition, the increase of psychological stress results in decreased serum levels of anti-Müllerian hormone in infertile women, implying that stress induction continuously may lead to amenorrhea, early menopause, and premature ovarian failure [56].

8.3.3 Immune Cell Activation

It is known that psychological pressure and stress promote pregnancy inflammation, including changes in the number of immune cells and increased secretion of inflammatory cytokines from these cells [9]. In general, the proportion of immune cells in uterine microenvironment is subtly changed after successful pregnancy, and inflammatory factors secreted by immune cells are slightly increased. But early pregnancy stress occurring on the first trimester can activate lymphocytes and promote high expression of cytokines, such as IL-1β and IL-6. A series of reactions can lead to an excessive inflammatory response and increase adverse pregnancy outcomes [57]. In addition, persistent stress in early pregnancy affects the proportion of Treg cells which cause Th1/Th2 cells unbalance. The current evidence shows that Th1 cells environment is harmful to embryo implantation and increases placental vascular resistance, which might cause preeclampsia, even lead to premature birth [58]. According to a large retrospective study, investigators detected the blood from specific kids whose mothers had experienced serious natural disasters (The 1998 Quebec ice storm) when they were pregnant. This survey found that the number of lymphocytes and CD4+ T cells was reduced while their cytokines significantly increased, such as TNF-α, IL-1β, and IL-6 levels. Stress can potentially alter the nervous and immune systems of offspring [59]. Neonatal immune system growth was regulated by the interaction between the nerve and endocrine system. Stress experienced by women in their early pregnancy permanently alters the fetal

respond ability of the nervous system. It affects the hypothalamic–pituitary–adrenal cortex (HPA) axis and alters the neural regulation of the immune system after birth subsequently [60].

8.3.4 Immune Regulatory Gene Expression and Modification

At present, there are few studies on the abnormal gene expression and modification induced by stress, and the specific mechanism is still unclear. Some scholars believe that the key mediator of stress is glucocorticoid (GC), which may affect the expression of related genes by binding to GC receptors, and possibly increase allergy susceptibility [61]. In the first trimester, neural stem cells have a rapid speed of self-renewal and proliferation. Then, they established synaptic connections in cerebrum. If this process is affected by stress, neural cells' proliferation and migration will be disordered, increasing the risk of neurological dysfunction. Then, these stress responses will be retained until birth through epigenetics, such as DNA methylation and histone modification. Finally, it's linked directly to schizophrenia and developmental disorders [62].

Oxidative stress (OS) is a physiological process of unbalance between oxidation and anti-oxidation. The main manifestation is neutrophil inflammatory infiltration and accumulation of oxidation intermediate products. OS is closely related to diseases of the female reproductive system, such as endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility, which directly leads to female reproductive failure [63]. Meanwhile, oxidative diseases are related to poor pregnancy outcomes, even spontaneous abortion and preeclampsia [64]. OS activates nuclear gene transcription through a variety of ROS (reactive oxygen species) sensitive components to regulate fetal development, including Nrf-2, NF-κB, and HIF-1 [63].

8.4 Outcomes of Stress-Induced Immune Response in Reproductive System

Various reports demonstrate that stress-induced immune response in pregnant female could lead to an abnormal increase of specific cytokines, resulting in fetal maldevelopment. Therefore, the alterations of immune response and the main clinical outcomes are briefly summarized here.

8.4.1 Alterations in Maternal Immune Function

Stress exposure has been shown to be capable of regulating both the maternal and offspring's immune function. We will briefly summarize these changes in recent studies from the aspects of immune cells, cytokines, and the immune-HPA axis.

Th1/Th2 and Th17/Treg immune balances are critical to maintain a successful pregnancy [37]. Literature reported that women with both severe depression (SD) and severe anxiety (SA) during the late pregnancy had the highest levels of Th1- (IL-6, TNF-α, IL-2, IFN-γ), Th17- (IL-17A, IL-22), and Th2- (IL-9, IL-10, and IL-13) related cytokines, and the SA group showed higher levels of Th1- (IL-6, TNF-α, IL-2, IFN-γ) and Th2- (IL-4, and IL-10) related cytokines than that of control group in serum [65].

Elevated stress can result in alterations of serum cytokine levels during pregnancy, leading to an alteration of maternal immune function. During early pregnancy, elevated stress is related to higher serum IL-6 and lower IL-10; during the second trimester of pregnancy, it is related to higher serum levels of C-reactive protein (CRP); elevated stress levels across pregnancy is related to an increased level of pro-inflammatory cytokines IL-1B and IL-6 [10, 66]. It is further confirmed by Giese S. et al that elevated stress is positively correlated with higher levels of the pro-inflammatory cytokines IL-6 and TNF-alpha, but is negetively correlated with lower levels of the anti-inflammatory cytokine IL-10 [67].

Interplays among stress, HPA axis, and immune system also contribute to the alteration of immune system after stress exposure. Stress exposure could lead to alteration of HPA axis as observed in animal studies [68]. The HPA axis can be activated by pro-inflammatory cytokines, resulting in glucocorticoid hormones release which in turn can deliver negative feedback and suppressed the cytokines release [69]. Lymphocyte sensitivity to corticosterone and catecholamines is altered under stress conditions, indicating adrenal's hormones are mediators of the differential reactions of stress on the immune response [48].

8.4.2 Reproductive Disorders: Female Infertility and Miscarriage, Preeclampsia, Recurrent Abortion

Despite maldevelopment of offspring, stress exposure might also result in reproductive disorders. Stress-related neural immune interactions may lead to various pregnancy complications and unsatisfactorily outcome [67]. Prenatal stress disorders caused maternal physiology and immune function disorder, leading to an increased risk of pregnancy complications such as preeclampsia and premature labor [10]. Although the relevance of stress exposure and pregnancy complications is not well documented, it is clear that pregnancy complications might arise more stress and stress exposure does have a negative effect on pregnancy.

8.4.3 Female Infertility and Miscarriage

Although it is accepted by some researchers that stress hampers reproductive function, more investigators believe that it is the infertility or other reproductive disorders that cause the psychological stress such as anxiety, depression, and irritability [70]. Previous study shows that psychological symptoms do have a negative effect on fertility [71], and oxidative stress is associated with female infertility, in that women suffered from stress get a higher miscarriage rate [72]. The relationship between placental oxidative stress and infertility still needs to be further studied. Compared to normal ones, transcriptomic analysis showed a decreased expression of genes in miscarriage placentas [73]. However, unexplained miscarriage samples are unable to be excluded, which makes it more difficult to study the relationship between stress and miscarriage.

8.4.4 Preeclampsia

Preeclampsia is one of the common pregnancy complications characterized by hypertension and proteinuria, which will lead to hypertension, edema, and eclampsia in mother and fetal growth restriction, prematurity, and death in baby. Oxidative and nitrosative stresses in placenta are reported to be associated with preeclampsia [74]. Oxidative stress in the placenta leads to inflammation and cellular apoptosis, and apoptotic cells will flow into maternal circulation and thus stimulate the release of more pro-inflammatory cytokines, resulting in a massive systemic endothelial dysfunction, even preeclampsia [74, 75]. Immune cells might contribute to pre-eclampsia by triggering stress in placenta. Immune cells found in placenta including dendritic cells, macrophages, and T cells are supposed to be involved in generating oxidative stress and might be also related to the onset of preeclampsia. Specifically, Hofbauer cells (placental macrophages) have been shown to express catalase at early pregnancy, which catalyze the detoxification of hydrogen peroxide (H_2O_2). Further study shows that Hofbauer cells might also induce nitrosative stress and cause other pathologies [76].

8.4.5 Recurrent Abortion

Women with recurrent abortion experience more psychological stress and major depression than pregnancy planners trying to conceive naturally [77]. However, stress exposure might also in turn lead to higher rate of recurrent abortion. It has been shown that oxidative stress is linked to recurrent abortion [78]. Oxidative stress-induced endothelial and placental vascularization impairment as well as immune malfunction may play an important role in the pathophysiology of recurrent abortion

[79]. However, how other stress exposure lead to a consequence of recurrent abortion requires more studies.

Collectively, stress exposure during pregnancy could bring adverse effects on both mother and offspring. From the mother's perspective, maternal stress exposure might result in mental disorders such as anxiety, aggression, and depression, even lead to preterm delivery and preeclampsia [80]. From the aspect of fetus or offspring, stress exposure is associated with premature labor and poor birth outcome. Although this conclusion is primarily based on animal studies, maternal stress exposure is undeniable to have a negative influence on both the mother and her fetus.

8.5 The Major Signal Pathways That Contribute to Stress-Induced Reproductive Immune Response in Females

8.5.1 Hypothalamic-Pituitary-Adrenal (HPA) Axis

Basic and clinical researches have shown that hypothalamic-pituitary-adrenal (HPA) axis plays a role in effect of stress on reproductive endocrine. Physical and psychological stresses can activate HPA axis, and affect female's reproductive endocrine function at all three aspects of the hypothalamo-pituitary-ovary (HPO) axis. Additionally, researches also reveal that the changes in progesterone and estrogen levels, and the different stages of one menstruation cycle, have impacts on the way females respond to stress.

The hyperfunction of HPA axis induced by stress can manifest as the excessive synthesis and secretion of corticotropin-releasing hormone (CRH) in paraventricular nucleus of the hypothalamus, promoting the secretion of adrenocorticotrophic hormone (ACTH) and beta endorphin (β-EP) in anterior pituitary, and ACTH acts on adrenal cortex to release glucocorticoids (GCS). Indeed, increased GCS level in plasma is often used as an objective indicator to judge stress reaction. Similar to HPA axis, there is a HPO axis in female. Gonadotropin-releasing hormone (GnRH) stimulates the synthesis and release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which trigger estradiol (E) and progesterone (P) secretion in ovaries. There is a feedback loop by which ovarian estradiol inhibits the release of GnRH and succeeding FSH and LH production. Alteration in the concentration of hormones stated above can affect the endocrine status of the HPO axis directly or indirectly. For example, hormones in HPA axis inhibit the GnRH secretion from the hypothalamus, and GCS inhibits LH secretion from the pituitary and estradiol and progesterone secretion from the ovary. In general, activation of the stress axis, especially repeating or chronic, has an inhibitory effect upon the release of gonadal hormone, and results in disorders of maternal immune function and infertility [81].

As one of the most important components of stress inhibiting reproductive endocrine, CRH is the major factor that inhibits the function of HPO axis. However, the molecular mechanisms are complex and unclear till now. Through in vitro experiments, researchers found that CRH inhibited GnRH secretion in the median eminence of the hypothalamus, and thus, we guessed there was direct synaptic connection between the terminal of axon of CRH and the dendrite of neurons secreting GnRH [82]. CRH may act directly on terminal of GnRH neuron to downregulate GnRH synthesis. Nevertheless, the inhibition of pituitary gonadotropin (Gn) release by corticotropin-releasing factor (CRF) is not mediated by ACTH, in that ACTH has no effect on the basic secretion and the release of LH from pituitary induced by LHRH. Furthermore, other studies suggest that β-endorphin bypass pathway may also involve in the effects on inhibition of CRH on GnRH [83].

Not only does CRH inhibit the secretion of Gn in pituitary, but it also inhibits ovarian estrogen synthesis directly. As FSH can promote estrogen synthesis in ovarian granulosa cells, CRH can inhibit this action by inhibiting aromatase which is essential in estrogen synthesis.

GCS are essential to the establishment and maintenance of reproductive function. Two apparent examples are two diseases with GCS disorder: Cushing's syndrome patients often complicate with reproductive disorders such as secondary amenorrhea in female. Similarly, patients with Addison's disease may suffer from premature ovarian failure in female or oligospermia in male. Researches reveal that increased GCS exposure, by either stress or exogenous treatment, can reduce the frequency and amplitude of LH significantly, especially near ovulation, and delay preovulatory LH and FSH surge, making E2 cannot reach the peak point, subsequently, leading to profound reproductive effects [84]. GCS modulate the HPO axis by inhibiting the release of GnRH in the hypothalamus as well as the synthesis and release of Gn in the pituitary directly. However, the exact signal pathways remain unclear yet. Some researchers argue that neuropeptides are key to study HPA function. CRH, thyrotropin-releasing hormone (TRH), oxytocin (OT), vasopressin (AVP), and some other hormones expressing neurons are among those that project to the median eminence [68]. Some of these neuropeptide neurons, such as kisspeptin (KISS1) and gonadotropin-inhibitory hormone (GnIH), are the drivers of the HPA axis. KISS1 neurons express glucocorticoid receptor (GR) in the anteroventral periventricular nucleus and periventricular nucleus continuum of the preoptic area of the hypothalamus, suggesting that GCS can directly act in neurons stated above. In the meanwhile, further studies reveal that to impair KISS1 neurons may be one of the mechanisms of GCS acting on HPO axis. Moreover, stress can increase the function of GnIH neurons, which can inhibit the neurons activity in both GnRH and KISS1 neurons, and increase contacts with GnRH neurons [85].

8.5.2 Epigenetic Pathway

Another signal pathways that contribute to stress-induced reproductive immune response in females is epigenetic pathway. Epigenetics describes how gene expression shows heritable changes without any change in DNA nucleotide sequence. Generally, the epigenetic signature can be passed on to the next generation and affect gene expression. The main regulation mechanisms of epigenetics include DNA methylation, histone modifications, chromatin remodeling, and non-coding RNA, among which DNA methylation is the one that has been most researched. For instance, DNA methylation is connected with gene silencing, while demethylation is linked to increased gene expression activity. Mostly, the activation of transcription at DNA level is the demethylation of the promoter sequence, while the promoter methylation inhibits transcription.

Large amount of evidence demonstrates the association of epigenetics and reproduction. *Kiss1* gene, as stated above, is discovered as a metastasis suppressor gene in malignant melanoma cell. Interestingly, it is then proved to be related to reproductive function in the aspect of the release of GnRH, onset of puberty, and also has functions on the maintenance of reproduction in adults [86]. Researches argue that the methylation of *Kiss1* and kisspeptin receptor (*Kiss1R*) genes promotes changes throughout puberty, and during puberty, the activation of *Kiss1* gene is the consequence of histone H3 modification activation [86]. Another example is the relationship between follicle maturation and chromatin remodeling. The aryl hydrocarbon receptor, Ahr, is regulated during ovarian follicle maturation, and its up-regulation relies on FSH and LH. The increase in Ahr protein is specifically related to large antral follicles in induced follicle maturation. Researches demonstrate that the activation of Ahr promoter can be regulated by chromatin remodeling, resulting in increased Ahr transcription [87]. In addition, Ahr in response to hCG is down-regulated by chromatin remodeling in preovulatory follicles in murine [87].

Recent studies reveal that DNA methylation is a possible mechanism of the effect of prenatal stress in the offspring. Prenatal stress may lead to aberrant heart structure, glucose intolerance, and brain function, etc. A study in America [88] studied whether stress and the absence of social support during pregnancy would affect maternal DNA methylation, and reached a conclusion that lack of support from family and friends, especially the baby's father, was associated with maternal DNA hypermethylation on multiple genes. Another study in 2017 [89] examined the multigenerational epigenetic effects of stress, especially psychosocial stress, and revealed that grandmaternal exposure to stress such as interpersonal violence during pregnancy was closely related to 27 differentially methylated CpG sites in children that mapped to 22 uniquely annotated genes. We take CORIN as an example, as for neonate. CORIN has functions on circulatory system processes and congenital abnormalities and as for prenatal disorders, it is associated with regulation of blood pressure and is important for physiological changes at the maternal–fetal interface. Animal studies have suggested that this gene plays a vital role in preventing gestational hypertensive disorder. Furthermore, methylation of another

gene, CFTR, may have relationship with depression symptoms and post-traumatic stress disorder (PTSD) [89].

Oxidative stress can also be a kind of stress in female reproduction. Oxidative and reductive stress can both have potentially hazardous effects. Oxidative stress has negative effects on reproductive function. Researches prove that it has been linked to female reproductive system diseases such as polycystic ovary syndrome and endometriosis [63]. As for epigenetics, reactive oxygen species (ROS) can cause DNA damage, which may affect the oocyte. Also, it may affect gametes quality and development of embryos. However, reactive oxygen species (ROS) are necessary for some physiological reproduction processes such as ovulation, capacitation, and corpus luteum formation and function. A regular example is ovulation. During an ovarian process, preovulatory LH surge emerge, ROS levels rise, and antioxidant levels fall [63].

8.6 Conclusions and Future Prospects

Stress exposure before and during pregnancy has capability to negatively affect both the mother and her fetus as reported by various human and animal models researches. One assumption accepted by most investigators indicate that maternal stress exposure might adversely affect the mother and her fetus via hormones and immune regulation. Therefore, it is promising to reduce maternal stress exposure to decrease the incidence of stress-induced physiological and psychological disorders. Hormones and immune regulation are also potential strategies through which we can lower the negative influence caused by stress-induced hormone or immune activation. Unfortunately, although specific cytokines and signal molecules are identified in both human and animals which suffered from pregnancy stress, the mechanisms by which maternal stress affect the mother and her fetus remain largely unknown. It is of great significance studying stress exposure during pregnancy since it has detrimental influence on maternal and child health with a high prevalence among pregnancy women worldwide. Further studies are still required to further elucidate the following issues: (1) How maternal stress triggers the activation of maternal immune system. (2) How different immune cells react to maternal stress. (3) How we can appropriately measure the maternal stress and establish a systemic stress management. (4) The mechanisms underlying the maldevelopment of the fetus and offspring. An insight into these issues will provide us more inspirations to successfully control maternal stress, providing the mother and her fetus a healthier physical and mental state.

References

1. Varcin KJ, Alvares GA, Uljarevic M, Whitehouse AJO. Prenatal maternal stress events and phenotypic outcomes in autism spectrum disorder. Autism Res. 2017;10:1866–77.
2. Li J, Vestergaard M, Obel C, Christensen J, Precht DH, et al. A nationwide study on the risk of autism after prenatal stress exposure to maternal bereavement. Pediatrics. 2009;123:1102–7.
3. Markham JA, Koenig JI. Prenatal stress: role in psychotic and depressive diseases. Psychopharmacology (Berl). 2011;214:89–106.
4. Gumusoglu SB, Fine RS, Murray SJ, Bittle JL, Stevens HE. The role of il-6 in neurodevelopment after prenatal stress. Brain Behav Immun. 2017;65:274–83.
5. Akatsu S, Ishikawa C, Takemura K, Ohtani A, Shiga T. Effects of prenatal stress and neonatal handling on anxiety, spatial learning and serotonergic system of male offspring mice. Neurosci Res. 2015;101:15–23.
6. Said N, Lakehayli S, Battas O, Hakkou F, Tazi A. Effects of prenatal stress on anxiety-like behavior and nociceptive response in rats. J Integr Neurosci. 2015;14:223–34.
7. Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, et al. Early life programming and neurodevelopmental disorders. Biol Psychiatry. 2010;68:314–9.
8. Avitsur R, Kavelaars A, Heijnen C, Sheridan JF. Social stress and the regulation of tumor necrosis factor-alpha secretion. Brain Behav Immun. 2005;19:311–7.
9. Christian LM. Psychoneuroimmunology in pregnancy: immune pathways linking stress with maternal health, adverse birth outcomes, and fetal development. Neurosci Biobehav Rev. 2012;36:350–61.
10. Coussons-Read ME, Okun ML, Nettles CD. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain Behav Immun. 2007;21:343–50.
11. Bogi E, Belovicova K, Moravcikova L, Csatlosova K, Dremencov E, et al. Pre-gestational stress impacts excitability of hippocampal cells in vitro and is associated with neurobehavioral alterations during adulthood. Behav Brain Res. 2019;375:112131.
12. Huang Y, Chen S, Xu H, Yu X, Lai H, et al. Pre-gestational stress alters stress-response of pubertal offspring rat in sexually dimorphic and hemispherically asymmetric manner. BMC Neurosci. 2013;14:67.
13. Mahmoodkhani M, Saboory E, Roshan-Milani S, Azizi N, Karimipour M, et al. Pregestational stress attenuated fertility rate in dams and increased seizure susceptibility in offspring. Epilepsy Behav. 2018;79:174–9.
14. Pluess M, Belsky J. Prenatal programming of postnatal plasticity? Dev Psychopathol. 2011;23:29–38.
15. Belsky J, Pluess M. Beyond diathesis stress: differential susceptibility to environmental influences. Psychol Bull. 2009;135:885–908.
16. Belsky J, Pluess M. Beyond risk, resilience, and dysregulation: phenotypic plasticity and human development. Dev Psychopathol. 2013;25:1243–61.
17. Hartman S, Freeman SM, Bales KL, Belsky J. Prenatal stress as a risk-and an opportunity-factor. Psychol Sci. 2018;29:572–80.
18. Glover V. Annual research review: prenatal stress and the origins of psychopathology: an evolutionary perspective. J Child Psychol Psychiatry. 2011;52:356–67.
19. Jafari Z, Mehla J, Kolb BE, Mohajerani MH. Prenatal noise stress impairs hpa axis and cognitive performance in mice. Sci Rep. 2017;7:10560.
20. Myers RE. Maternal psychological stress and fetal asphyxia: a study in the monkey. Am J Obstet Gynecol. 1975;122:47–59.
21. Anderson DK, Rhees RW, Fleming DE. Effects of prenatal stress on differentiation of the sexually dimorphic nucleus of the preoptic area (sdn-poa) of the rat brain. Brain Res. 1985;332:113–8.
22. Al-Ayadhi LY, Mostafa GA. Elevated serum levels of interleukin-17a in children with autism. J Neuroinflammation. 2012;9:158.

23. Petitto JM, McCarthy DB, Rinker CM, Huang Z, Getty T. Modulation of behavioral and neurochemical measures of forebrain dopamine function in mice by species-specific interleukin-2. J Neuroimmunol. 1997;73:183–90.
24. Gilmore JH, Fredrik Jarskog L, Vadlamudi S, Lauder JM. Prenatal infection and risk for schizophrenia: Il-1beta, il-6, and tnfalpha inhibit cortical neuron dendrite development. Neuropsychopharmacoly. 2004;29:1221–9.
25. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci Off J Soc Neurosci. 2007;27:10695–702.
26. Wu WL, Hsiao EY, Yan Z, Mazmanian SK, Patterson PH. The placental interleukin-6 signaling controls fetal brain development and behavior. Brain Behav Immun. 2017;62:11–23.
27. Mouihate A, Mehdawi H. Toll-like receptor 4-mediated immune stress in pregnant rats activates stat3 in the fetal brain: role of interleukin-6. Pediatr Res. 2016;79:781–7.
28. Voorhees JL, Tarr AJ, Wohleb ES, Godbout JP, Mo X, et al. Prolonged restraint stress increases il-6, reduces il-10, and causes persistent depressive-like behavior that is reversed by recombinant il-10. PLoS One. 2013;8:e58488.
29. Pace TW, Mletzko TC, Alagbe O, Musselman DL, Nemeroff CB, et al. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. Am J Psychiatry. 2006;163:1630–3.
30. Tuchscherer M, Kanitz E, Otten W, Tuchscherer A. Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. Vet Immunol Immunopathol. 2002;86:195–203.
31. Mahmoud F, Abul H, Dashti A, Al-Jassar W, Omu A. Trace elements and cell-mediated immunity in gestational and pre-gestational diabetes mellitus at third trimester of pregnancy. Acta Medica Academica. 2012;41:175–85.
32. Horn SR, Roos LE, Berkman ET, Fisher PA. Neuroendocrine and immune pathways from pre- and perinatal stress to substance abuse. Neurobiol Stress. 2018;9:140–50.
33. Zakharova LA. Perinatal stress in brain programming and pathogenesis of psychoneurological disorders. Izv Akad Nauk Ser Biol. 2015:17–26.
34. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci. 2009;10:434–45.
35. Csaba G, Tekes K, Pallinger E. Influence of perinatal stress on the hormone content in immune cells of adult rats: dominance of acth. Hormone Metab Res Hormon- und Stoffwechselforschung/Hormones et metabolisme. 2009;41:617–20.
36. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16:22–34.
37. Leff-Gelman P, Mancilla-Herrera I, Flores-Ramos M, Cruz-Fuentes C, Reyes-Grajeda JP, et al. The immune system and the role of inflammation in perinatal depression. Neurosci Bull. 2016;32:398–420.
38. Kaufman J, Plotsky PM, Nemeroff CB, Charney DS. Effects of early adverse experiences on brain structure and function: clinical implications. Biol Psychiatry. 2000;48:778–90.
39. Avitsur R, Maayan R, Weizman A. Neonatal stress modulates sickness behavior: role for proinflammatory cytokines. J Neuroimmunol. 2013;257:59–66.
40. Avitsur R, Sheridan JF. Neonatal stress modulates sickness behavior. Brain Behav Immun. 2009;23:977–85.
41. Ackerman SH, Keller SE, Schleifer SJ, Shindledecker RD, Camerino M, et al. Premature maternal separation and lymphocyte function. Brain Behav Immun. 1988;2:161–5.
42. Laudenslager M, Capitanio JP, Reite M. Possible effects of early separation experiences on subsequent immune function in adult macaque monkeys. Am J Psychiatry. 1985;142:862–4.
43. Teunis MA, Heijnen CJ, Sluyter F, Bakker JM, Van Dam AM, et al. Maternal deprivation of rat pups increases clinical symptoms of experimental autoimmune encephalomyelitis at adult age. J Neuroimmunol. 2002;133:30–8.
44. Kruschinski C, Skripuletz T, Bedoui S, Raber K, Straub RH, et al. Postnatal life events affect the severity of asthmatic airway inflammation in the adult rat. J Immunol. 2008;180:3919–25.

45. Michaut RJ, Dechambre RP, Doumerc S, Lesourd B, Devillechabrolle A, et al. Influence of early maternal deprivation on adult humoral immune response in mice. Physiol Behav. 1981;26:189–91.
46. Schmidt MV, Enthoven L, van der Mark M, Levine S, de Kloet ER, et al. The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse. International journal of developmental neuroscience: the official journal of the international society for. Dev Neurosci. 2003;21:125–32.
47. Meaney MJ, Diorio J, Francis D, Widdowson J, LaPlante P, et al. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. Dev Neurosci. 1996;18:49–72.
48. Silberman DM, Wald MR, Genaro AM. Acute and chronic stress exert opposing effects on antibody responses associated with changes in stress hormone regulation of t-lymphocyte reactivity. J Neuroimmunol. 2003;144:53–60.
49. Merlot E, Couret D, Otten W. Prenatal stress, fetal imprinting and immunity. Brain Behav Immun. 2008;22:42–51.
50. Blackmore ER, Moynihan JA, Rubinow DR, Pressman EK, Gilchrist M, et al. Psychiatric symptoms and proinflammatory cytokines in pregnancy. Psychosom Med. 2011;73:656–63.
51. Haimovici F, Anderson JL, Bates GW, Racowsky C, Ginsburg ES, et al. Stress, anxiety, and depression of both partners in infertile couples are associated with cytokine levels and adverse ivf outcome. Am J Reprod Immunol. 2018;79:e12832.
52. Valsamakis G, Papatheodorou DC, Chalarakis N, Vrachnis N, Sidiropoulou EJ, et al. In pregnancy increased maternal stai trait stress score shows decreased insulin sensitivity and increased stress hormones. Psychoneuroendocrinology. 2017;84:11–6.
53. Weinstock M. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav Immun. 2005;19:296–308.
54. Schliep KC, Mumford SL, Vladutiu CJ, Ahrens KA, Perkins NJ, et al. Perceived stress, reproductive hormones, and ovulatory function: a prospective cohort study. Epidemiology. 2015;26:177–84.
55. Lynch CD, Sundaram R, Buck Louis GM. Biomarkers of preconception stress and the incidence of pregnancy loss. Hum Reprod. 2018;33:728–35.
56. Dong YZ, Zhou FJ, Sun YP. Psychological stress is related to a decrease of serum anti-mullerian hormone level in infertile women. Reprod Biol Endocrinol. 2017;15:51.
57. Christian LM. Effects of stress and depression on inflammatory immune parameters in pregnancy. Am J Obstet Gynecol. 2014;211:275–7.
58. Schminkey DL, Groer M. Imitating a stress response: a new hypothesis about the innate immune system's role in pregnancy. Med Hypotheses. 2014;82:721–9.
59. Bellinger DL, Lubahn C, Lorton D. Maternal and early life stress effects on immune function: relevance to immunotoxicology. J Immunotoxicol. 2008;5:419–44.
60. Kisanga EP, Tang Z, Guller S, Whirledge S. Glucocorticoid signaling regulates cell invasion and migration in the human first-trimester trophoblast cell line sw 71. Am J Reprod Immunol. 2018;80:e12974.
61. Moustaki M, Tsabouri S, Priftis KN, Douros K. Prenatal stress enhances susceptibility to allergic diseases of offspring. Endocr Metab Immune Disord Drug Targets. 2017;17:255–63.
62. Udagawa J, Hino K. Impact of maternal stress in pregnancy on brain function of the offspring. Nihon Eiseigaku Zasshi Japanese J Hygiene. 2016;71:188–94.
63. Menezo YJ, Silvestris E, Dale B, Elder K. Oxidative stress and alterations in DNA methylation: two sides of the same coin in reproduction. Reprod Biomed Online. 2016;33:668–83.
64. Wu F, Tian FJ, Lin Y, Xu WM. Oxidative stress: placenta function and dysfunction. Am J Reprod Immunol. 2016;76:258–71.
65. Leff Gelman P, Mancilla-Herrera I, Flores-Ramos M, Saravia Takashima MF, Cruz Coronel FM, et al. The cytokine profile of women with severe anxiety and depression during pregnancy. BMC Psychiatry. 2019;19:104.

66. Cheng CY, Pickler RH. Perinatal stress, fatigue, depressive symptoms, and immune modulation in late pregnancy and one month postpartum. Sci World J. 2014;2014:652630.
67. Coussons-Read ME, Okun ML, Schmitt MP, Giese S. Prenatal stress alters cytokine levels in a manner that may endanger human pregnancy. Psychosom Med. 2005;67:625–31.
68. Oyola MG, Handa RJ. Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. Stress. 2017;20:476–94.
69. Bateman A, Singh A, Kral T, Solomon S. The immune-hypothalamic-pituitary-adrenal axis. Endocr Rev. 1989;10:92–112.
70. Rooney KL, Domar AD. The relationship between stress and infertility. Dialogues Clin Neurosci. 2018;20:41–7.
71. Rooney KL, Domar AD. The impact of stress on fertility treatment. Curr Opin Obstet Gynecol. 2016;28:198–201.
72. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol. 2012;10:49.
73. Lyu SW, Song H, Yoon JA, Chin MU, Sung SR, et al. Transcriptional profiling with a pathway-oriented analysis in the placental villi of unexplained miscarriage. Placenta. 2013;34:133–40.
74. Aouache R, Biquard L, Vaiman D, Miralles F. Oxidative stress in preeclampsia and placental diseases. Int J Mol Sci. 2018;19:1496.
75. Goulopoulou S, Davidge ST. Molecular mechanisms of maternal vascular dysfunction in preeclampsia. Trends Mol Med. 2015;21:88–97.
76. Sisino G, Bouckenooghe T, Aurientis S, Fontaine P, Storme L, et al. Diabetes during pregnancy influences hofbauer cells, a subtype of placental macrophages, to acquire a pro-inflammatory phenotype. Biochim Biophys Acta. 2013;1832:1959–68.
77. Kolte AM, Olsen LR, Mikkelsen EM, Christiansen OB, Nielsen HS. Depression and emotional stress is highly prevalent among women with recurrent pregnancy loss. Hum Reprod. 2015;30:777–82.
78. Ishii T, Miyazawa M, Takanashi Y, Tanigawa M, Yasuda K, et al. Genetically induced oxidative stress in mice causes thrombocytosis, splenomegaly and placental angiodysplasia that leads to recurrent abortion. Redox Biol. 2014;2:679–85.
79. Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. Obstet Gynecol Surv. 2007;62:335–47.
80. Field T, Diego M, Hernandez-Reif M. Prenatal depression effects on the fetus and newborn: a review. Infant Behav Dev. 2006;29:445–55.
81. Toufexis D, Rivarola MA, Lara H, Viau V. Stress and the reproductive axis. J Neuroendocrinol. 2014;26:573–86.
82. Smith RF, Ghuman SP, Evans NP, Karsch FJ, Dobson H. Stress and the control of lh secretion in the ewe. Reproduction. 2003;61:267–82.
83. Tellam DJ, Mohammad YN, Lovejoy DA. Molecular integration of hypothalamo-pituitary-adrenal axis-related neurohormones on the gnrh neuron. Biochem Cell Biol. 2000;78:205–16.
84. Breen KM, Billings HJ, Wagenmaker ER, Wessinger EW, Karsch FJ. Endocrine basis for disruptive effects of cortisol on preovulatory events. Endocrinology. 2005;146:2107–15.
85. Whirledge S, Cidlowski JA. Glucocorticoids and reproduction: traffic control on the road to reproduction. Trends Endocrinol Metab. 2017;28:399–415.
86. Motti ML, Meccariello R. Minireview: the epigenetic modulation of *kiss1* in reproduction and cancer. Int J Environ Res Public Health. 2019;16:2607.
87. Matvere A, Teino I, Varik I, Kuuse S, Tiido T, et al. Fsh/lh-dependent upregulation of ahr in murine granulosa cells is controlled by pka signaling and involves epigenetic regulation. Int J Mol Sci. 2019;20:3068.

88. Surkan PJ, Hong X, Zhang B, Nawa N, Ji H, et al. Can social support during pregnancy affect maternal DNA methylation? Findings from a cohort of African-Americans. Pediatr Res. 2019;88:131–8.
89. Serpeloni F, Radtke K, de Assis SG, Henning F, Natt D, et al. Grandmaternal stress during pregnancy and DNA methylation of the third generation: an epigenome-wide association study. Transl Psychiatry. 2017;7:e1202.

Chapter 9
Effects of Environmental EDCs on Oocyte Quality, Embryo Development, and the Outcome in Human IVF Process

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Abstract In our daily life, people are inevitably exposed to potentially hazardous chemical contaminants. More and more evidences indicate that environmental endocrine-disrupting chemicals (EDCs) negatively affect human reproductive health and are related to many diseases including infertility. Environmental reproductive health focuses on exposure to ubiquitous and persistent EDCs. This chapter mainly discusses the effects of EDCs on the outcome of human in vitro fertilization (IVF), including oocyte quality, fertilization, embryo quality, implantation, and live births. It may be useful for doctors to advise IVF patients to avoid these adverse environmental factors as much as possible. In addition, it is important for clinical embryologists to bear in mind that adverse IVF outcome may result from such undesirable environmental exposure, and quality management and quality control in the IVF laboratory should be strengthened.

Keywords Oocyte · Embryo · EDCs · Human IVF

9.1 Introduction of EDCs with Infertility

Since the beginning of the twenty-first century, the incidence of human infertility in many countries has increased more than people expected [1, 2]. The growth rate of infertility is too rapid to be explained simply by genetic mutations. In China, more than 10% of infertile couples suffer from unexplained infertility. The women in these couples have neither obvious reproductive organ diseases nor abnormal ovulatory cycles and hormonal levels, and the semen quality of their partners was not obviously abnormal. Despite substantial efforts to define the cause of infertility, the underlying mechanism has not been fully elucidated [3]. With the increasing production of numerous artificial chemicals and environmental pollution, people are

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exposed to potentially hazardous chemical contaminants in their daily life [4]. At present, there is imminent concern that these increased hazardous chemical contaminants have been associated with poor fertility in women.

Contaminants exposed to humans can be largely categorized into natural and artificial exposure. As such, they have been collectively defined as endocrine-disrupting chemicals (EDCs). Studies have shown that EDCs can mimic endogenous hormones or competitive binding with their receptors, thereby exerting a series of biological functions, adversely affect reproductive health owing to its widespread exposure and endocrine-disrupting properties [4].EDCs interfere with the production, secretion, transport, metabolism, binding, excretion of natural hormones in the body [5]. Precocious puberty, ovulation disorders, infertility, and other reproductive related diseases may be caused by these effects [6]. In the relatively short time that sensitive biomarkers for assaying EDCs exposure in humans have been available, a rapidly growing amount of literature has demonstrated that human exposure is ubiquitous, and evidence for adverse impacts on human reproductive health is mounting [7, 8].

Female infertility is a complex disease and can be caused by many factors including genetic, environmental, and behavioral [9]. Generally, human genome is relatively stable at the population level, so environmental and lifestyle-related factors may play a more important role in the increase of infertility. More and more literatures shown that EDCs exposure are related to gynecologic tumor, endometriosis, polycystic ovary syndrome (PCOS), premature ovarian failure (POF), and other diseases [10]. Both epidemiological and experimental evidence demonstrate that EDCs affect reproduction-related gene expression and epigenetic modification that are closely associated with infertility. Therefore, the detrimental effects of EDCs on reproduction may be lifelong and transgenerational [11–13].

In China, since the early 1990s, the increasing use of assisted reproductive technology (ART), including in vitro fertilization (IVF), has offered us a ready source of oocytes and follicular fluid (FF), promoting interest in research of the impact of EDCs on female fertility. The follicle is a very delicate microenvironment in which an oocyte with developmental potential is developed by interacting with their surrounding granular cells, hormones, growth factors, macromolecules, and other proteins [14]. ART procedures provide valuable opportunities to explore the connections between environmental contaminants in the ovarian microenvironment and measures of fertility. Due to ethical issues, it is difficult to launch mechanism researches on human oocyte [15]. However, biomarkers of environmental exposure may be measured in FF or blood. ART provides the opportunity to explore stages of reproduction that are otherwise not observable in the general population, such as oocyte quality, fertilization, embryo quality, and implantation [16, 17]. The higher the level of EDCs contamination in the follicular microenvironment, the lower the fertilization rate and the lower the rate of high-quality embryos [18].

Environmental reproductive health focuses on exposure to ubiquitous and persistent EDCs, especially those with similar molecular structure to endogenous estrogen, progesterone, and luteinizing hormone. EDCs are associated with altered reproductive health [5]. As a threat to modern civilization, EDCs, can significantly

extend the time to pregnancy, although rarely leading to irreversible infertility [19, 20]. Therefore, many infertile couples have to choose IVF. Gametes and embryos are sensitive to EDCs in their environment, which is one of the vital factors affecting embryonic development. This chapter mainly discusses the effects of EDCs on the outcome of human IVF, including oocyte quality, fertilization rate, embryo quality, implantation, and live births. The sort of EDCs discussed, respectively, is as follows: bisphenol A (BPA), volatile organic compounds (VOCs), triclosan, phthalates, parabens, perfluorinated compounds (PFCs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs). This chapter may be useful to advise IVF patients to avoid these adverse environmental factors as much as possible.

9.2 EDCs and Effects on Human IVF Outcomes

9.2.1 Oocyte Quality

Oocytes arise from the primordial germ cells during development of the fetus. After DNA replication, accompanying the occurrence of chromosomes condense and undergo recombination, oocytes enter the prophase of the first meiotic division (prophase I). The follicles at this time are called primordial follicles, with the chromosomes dispersed and the oocytes are surrounded by a single layer of granulosa cells. Oocytes remain arrested at prophase I, at the germinal vesicle (GV) stage, until puberty. During every menstrual cycle, oocytes and follicles enter the growth phase and gradually resume meiosis in response to LH surge. The first sign of the resumption of meiosis is germinal vesicle breakdown (GVBD), followed by metaphase I, anaphase I, telophase I, and metaphase II.

Within the reproductive system, the ovarian follicle can be supposed as an extremely fragile microenvironment, in which interactions between hormones, growth factors, the oocyte and its surrounding granular cells are indispensable to develop a fully competent oocyte [21–23]. Disruption of this finely tuned (endocrine/paracrine) balance can lead to a diminished oocyte quality which harms further embryo development [24]. In ART clinical practice, the top quality oocytes are defined as those with round appearance, a smooth first polar body, good refraction, normal perivitelline space (PVS), normal zona pellucida (ZP), and without cytoplasmic defects such as vacuoles, refractile bodies, central granulation, and smooth endoplasmic reticulum. EDCs have various effects on the ovary and, besides, it is possible that a certain stage of oocyte development especially vulnerable to EDCs, including meiotic initiation in the fetal ovary, follicle formation in the perinatal period, and oocyte growth and resumption of meiosis in the adult [7].

9.2.1.1 BPA

It is likely that BPA is an ovarian toxicant and acts via numerous pathways including oxidative stress, apoptosis, and folliculogenesis. Both epidemiological and experimental evidence demonstrate that all bisphenol affect female infertility and/or subfertility [25, 26].

Human exposure to BPA is nearly ubiquitous through inhalation, ingestion, and dermal absorption. BPA is detected in the urine of more than 90% of participants, in the interim found in other human fluids including blood, amniotic fluid, and in FF [27]. A research measured serum BPA levels in patients undergoing IVF-ET, then correlated them with oocyte maturation and fertilization results, elucidating that BPA had a troubled influence on oocyte quality and embryo development [28]. Moreover, in human, BPA exposure has a dose-response association with altered oocyte maturation in vitro [29]. Human data are even limited, however, studies have shown that humans were more sensitive than animals to the behavioral effects of BPA. Animal data have demonstrated that BPA exposure is related to oocyte maturation arrest and microtubule spindle assembled abnormalities. BPA exposure is associated with increased oocyte aneuploidy, as well as harmful to meiotic centrosome dynamics, spindle formation, even chromosome alignment and segregation [30, 31]. In another *in vitro* study, prolonged exposure to low concentrations of BPA reported a non-linear dose-dependent effect on the meiotic spindle with perturbation in chromosome alignment in MII oocytes [32]. In animal models, BPA has adverse effects on oocyte meiosis, interferes with GVBD, decreases the primordial follicle pool via stimulating the initial recruitment and subsequent follicle development until the antral stage, disturbs ovarian steroidogenesis, modifies normal uterine morphology, and impairs uterine receptivity and embryo implantation [26].

However, there has very little useful data concerning potential exposure of oocytes to BPA during IVF in human, meanwhile, people are paying more and more attention to whether BPA exposure exists in IVF process [33]. Because of the ubiquitous presence of BPA in plastics, proprietary information regarding the coatings used in the IVF culture dishes, most embryologists are more concerned about whether BPA is measurable in IVF laboratory, including culture media and tissue culture plastic products [34]. So far, there are many plastic-related chemicals with endocrine activity that may also be in the leachate. It is certainly possible that oocytes, sperms, and embryos are subsequently exposed to BPA leached from the culture dishes or aspiration tubing tested directly under normal-use conditions [35]. Fortunately, there has already been an experimental study indicated that detectable concentrations of BPA were not detected in daily used culture media, culture dishes, or suction tubing [35].

9.2.1.2 VOCs

Nowadays VOCs widely used in industrial products and processes [36]. Rapid urbanization and industrialization have been linked to the growth of VOC emissions [37]. Most emitted VOCs can result in the generation of secondary pollutants, the toxic and carcinogenic human health effects of which are detailed reported [38, 39].

It is generally known that VOCs are detrimental for IVF outcomes, because VOCs are hydrocarbon-based organic compounds that have a high vapor pressure at room temperature [40–43]. Unfortunately, lots of materials can release numerous VOCs in IVF lab, including PVC flooring materials, cosmetics and even autoclaved instrument when packs are opened for use [44, 45]. Presently, ethanol remains to be a disinfectant in some IVF laboratories because of its broad effect against bacteria, what people neglect, however, is that it is also a known VOC [44]. Studies have shown that EDCs including VOCs can have detrimental effects on IVF outcomes by affecting the quality of gametes and embryos in IVF laboratory. Apart from in conjunction with the developmental functions of gap junctions in the ovary, gap junctions serve as one major communication ways between oocyte and somatic cells, which are good targets for reproductive toxicants. These reproductive toxicants may also target at gap junction intercellular communication (GJIC), another conduits for toxicants to reach the oocyte. The breakdown of GJIC in virtue of ovotoxicant exposure is a potential etiology influencing the quality of oocytes [46]. Embryonic growth and development are affected by direct attachment of VOCs to embryonic DNA and terminating its growth. Moreover, in human sperm VOCs are also closely related to DNA fragmentation, which may cause sperm DNA damage and subsequently the number of male-mediated infertility, miscarriage, and other adverse reproductive outcomes have risen [47].

9.2.1.3 Triclosan

Triclosan (TCS) is a broad-spectrum antimicrobial agent being widely applied in pharmaceuticals and personal care products, especially with a molecular structure similarly to anthropogenic estrogens. There is no suggestion TCS had been regarded as a chemical pollutant previously, resulting in widespread use without proper regulation [48]. However, compared to other EDCs, we know far less regarding the impact of TCS as one of the "emerging EDCs of interest" [49]. Since its ubiquitous existence in the environment and human population lately, the reproductive endocrine-disrupting effects of TCS may be mediated either through sex hormone-related pathways or during steroidogenesis [50]. Supposing that the mother is exposed during pregnancy, the potential toxic effects of TCS may influence the health of offspring [51].

So far, there have been only a few studies that assessed the direct relation between urinary TCS concentration and early reproductive outcomes, as well as its endocrine-disrupting effects among women seeking fertility treatment, and the results found a

correlation between urinary triclosan concentrations and oocyte yield [52]. In addition, the synergistic effect of TCS with BPA and ethinylestradiol indicates its disruptive effects in sex hormone metabolism, while subtle changes in hormone levels can influence oocyte development [8, 53]. Furthermore, according to the time of exposure the sensitivity of the female reproductive system to BPA and TCS could change, which may affect the quality of oocytes [54].

9.2.1.4 Phthalates

Phthalates are high-production-volume synthetic chemicals with ubiquitous human exposure because of their use in plastics and other common consumer products [55]. Recent epidemiologic evidence shows that women have a unique exposure profile to phthalates, which raises concern about the potential health hazards posed by such exposure. For specific high-risk group women, phthalates exposure is predominantly via cosmetics and food [56]. In humans, it pays more and more attention that exposure to phthalates possibly produced harmful effects during development [57]. Phthalates cross from maternal blood into the developing fetus via placental transfer and into neonates via breast milk, and the exposure may affect the developing endocrine system, which is essential for diverse biological functions, including sexual development and reproductive functions in adults [58].

Urine is the preferred matrix for phthalate determination in humans [59]. A study was to evaluate whether urinary concentrations of metabolites of phthalates and phthalate alternatives were associated with intermediate and clinical IVF outcomes, and the results showed that several phthalate biomarkers were inversely associated with the number of total oocytes, mature oocytes yield, fertilized oocytes, and top quality embryos. In short, these phthalates can influence early IVF outcomes, specifically oocyte parameters [60]. Karwacka et al. estimated epidemiological studies conducted within the last 16 years and showed evidence that the antral follicle count, oocyte quality, implantation, the rate of clinical pregnancy and live birth were decreased after exposure to phthalates [7].

Even low concentrations of phthalate can impair the follicle-enclosed oocyte. Researchers, using the bovine model, provided evidence of impaired oocyte developmental capacity, manifested by a decrease in fertilizaiton rates and the proportion of available embryos [61]. Moreover, blastocysts that developed from phthalate-treated oocytes had impaired transcript abundance, suggesting their low quality [61, 62]. Similarly, further study in mice expands our understanding on how phthalates affect reproduction, and it turned out that phthalate exposure can induce oxidative stress as well as inhibit growth in antral follicles [63].

9.2.1.5 Parabens

Parabens are a group of alkyl esters of *p*-hydroxybenzoic acid which are widely used as preservatives in food, pharmaceuticals, cosmetics, and personal care products

[64, 65]. In fact, because of the use of these estrogenic chemicals in many cosmetics, exposure to parabens is more common in women than in men [66, 67]. Despite available data showing widespread women exposure to parabens, there are a limited number of studies, both in animals and human, on the association between parabens and female reproductive disease. A suggested trend of decreased antral follicle count, accompanied by the decrease of oocytes number, was observed correlation to increased levels of propyl parabens in women undergoing IVF [68].

However, humans are typically exposed to many man-made chemicals simultaneously. Thus, investigating one chemical at a time may not represent the effect of mixtures [69]. Recent studies have shown that the fact that exposed to EDCs concurrently was harmful to a healthy pregnancy is more crucial than previously thought [70]. Also, some studies show that the mixtures of parabens and phenols may impact ovarian hormone levels [65, 70]. The mixtures can affect hormone levels through up-regulating ERα or by disrupting cholesterol transport, further influencing steroid hormone synthesis, but beyond that, ERβ might be preferentially bound by parabens [71].

Similarly, recent studies have estimated the association between urinary paraben concentrations and early IVF outcomes, and shown that there were no significant correlations of methyl (MP), propyl (PP), and butyl paraben (BP) with number of oocytes retrieved, oocyte maturity, cleavage rate, blastocyst formation, or implantation, but increased urinary MP and PP were associated with increased incidence of poor embryo quality [72]. However, beyond impacting embryo quality, PP and MP were not associated with fertilization or live birth [73]. In any case, current investigations support that low-level exposure to parabens may play a part in changing reproductive hormone levels, with potential subsequent implications for the outcome of IVF. Nevertheless, additional toxicological and mechanistic researches are needed to better explore potential effects on female reproduction, if any, of parabens [73].

9.2.1.6 Perfluorinated Compounds

PFCs are man-made compounds produced industrially by electrochemical fluorination, and are world widely used as emulsifiers in cleaning products, as inert components in shampoos, food containers, pesticides, etc. [7, 74] Because of their high stability, PFCs are persistent, ubiquitous, and bioaccumulate in the environment [75]. While their acute toxicity is not high, the first and most stubborn problem is their stability and their ability to persist, and thus one of the most commonly studied PFCs and perfluorooctane sulfonate (PFOS) has been listed as a persistent organic pollutant under the Stockholm Convention since May 2009 [76]. Surprisingly, while many researches have suggested the correlations between exposure to PFCs and reproductive functions both in animal and human studies, the direct impact about PFCs on female fertility has been scarcely studied [74, 75, 77]. Moreover, the biological mechanisms by which exposure to PFCs influences upon fertility are unknown.

It is well known that conventional overnight fertilization exposes oocytes, spermatozoa, and zygotes to potentially deleterious substances in a concentration-dependent manner. For example, mineral oil containing cumene hydroperoxide has been shown to undergo peroxidation under certain storage conditions in IVF-ET, and cumene hydroperoxide in mineral oil is found to decrease blastocyte cell number in a dose-dependent manner [78]. To evaluate the mechanisms of oocyte maturation inhibition by PFCs, the effects of PFCs during porcine oocyte maturation, GJIC between oocytes and granulosa cells have been assessed. GJIC between oocytes and granulosa cells is critically affected during the first 8 h of oocyte maturation, and the inhibition of GJIC by PFCs in a dose-dependent manner [79, 80]. These findings suggest that PFCs may influence oocyte maturation by disturbing the GJIC in the cumulus–oocyte complexes (COCs) during the first several hours of maturation, and more seriously, it may be dangerous to occupationally exposed populations. In addition, oocytes are more sensitive than other cell types to the effect of PFCs [77, 80].

And finally, it must be pointed out that there has been already a study examined in human undergoing IVF-ET cycles, which detected PFCs contamination in FF and assessed the effect of PFCs levels on oocyte quality and fertilization rate. The current investigation results showed a significant correlation between FF PFC levels and IVF outcomes [81]. Researchers' explanation was the number of embryos transferred reduced owing to a harmful effect of PFC on oocytes fertilization capacity. A big drawback in this study, however, was its only 16 patients sample, which could not display of any significant differences in oocytes quality or pregnancy rate. This preliminary study need to confirm by some larger sample size studies with a concomitant increased awareness to PFCs threat on female fertility. This potential exposure risk should raise concerns over the female reproductive toxicity of these chemicals [81, 82].

9.2.1.7 PCBs and OCPs

Many products and byproducts of manufacturing processes are supposed to be potential causes of endocrine defects. There are currently more than one-fourth are persistent and lipophilic OCPs in 70,000 such chemicals on the market [83]. In many developed countries, due to their confirmed detrimental effect on ecosystem and human health, PCBs and OCPs have been restricted. Nevertheless, both of them could be checked as before in our environment on account of their long half-lives. In China, a study investigated concentrations of 17 OCPs in 127 infertile female's FF undergoing IVF treatment, suggesting a wide concentration range of OCPs in FF [84]. Furthermore, EDCs suffer the process of bioaccumulation because of their lipophilic and persistent distinguishing feature, with the highest concentrations found in species at the top of the food chain [85]. Chlorinated biphenyl 153 and p, p′-DDE are the compounds detected in the highest concentrations in FF and serum samples, respectively [86].

Using the pig as a toxicological model, researchers evaluated that exposing immature COCs to an organochlorine mixture during in vitro maturation (IVM) would detrimentally influence oocyte maturation, fertilization, and subsequent embryo development. COCs were cultured in IVM medium containing high level of the organochlorine mixture, similar to that found in women of highly exposed populations [87, 88]. This culture course mimicked the routine in IVF laboratory culture processes. The results demonstrated that OCPs lessened the quality of cumulus expansion and the viability of cumulus cells in a dose-response manner, as well as blastocyst formation and number of cells per blastocyst decreased with organochlorine concentration. Exposing porcine COCs to an environmentally relevant organochlorine mixture during IVM affects oocyte development, supporting continuously concerns that OCPs impair reproductive health in humans and other mammalian species. In addition, other studies have demonstrated that the ability of PCBs to interfere with the organization of the microtubules cytoplasmic network leading to an altered compartmentalization of the ooplasm [85].

9.2.2 Fertilization, Embryo Quality, Implantation, and Live Birth

From the maturation of an oocyte to a viable fetus, their biological characteristics have changed dramatically. The levels of various sex hormones, such as progesterone, estrogen, and luteinizing hormone, as well as their receptors also change steadily [54]. The success of implantation depends on the complex molecular interactions between the vital blastocysts and the receptive uterus. The complex molecular interactions between the uterus and the blastocyst require a carefully synchronized hormone signals and feedback loops, at which stage they may be vulnerable to chemicals such as EDCs, which may disrupt endocrine signaling [89]. Thus, the sensitivity of the female reproductive system to EDCs may vary depending on the time of exposure [54]. The higher level of EDCs contamination in follicles, the lower the fertilization rate, and the lower chance of oocytes developing into top quality embryos [18].

Human chorionic gonadotropin (hCG) is initially produced by trophoblast cells of embryos, which rises rapidly after embryo implantation [20]. The abrupt rise of hCG in early stages of pregnancy can be used to identify the timing of implantation of the embryo [33].In IVF cycle, 14 days after embryo transfer, when the hCG levels in the blood of the patient is detected to be lower than 5.0 mIU/mL, the cycle outcome is defined as a failure of embryo implantation. In women who attempt to conceive naturally, implant failure may be manifested in reduced fertility and prolonged pregnancy, as it is often difficult to determine the exact time of embryo fertilization as in IVF process. Implant failure is the most common failure point in IVF cycle, so which is often chosen as the observation point for research. Studies have shown that

many EDCs can be detected in FF, and EDCs level in FF may be related to embryo implantation failure [18].

These prompt embryologists to select the top grade of IVF-specific products, such as culture media and labware, instrumentation including LASERs, low oxygen incubators, and tools such as time-lapse monitoring system for embryo assessment [90]. It has been proved that laboratory environment is crucial for the embryo quality because of the presence of VOCs, BPA, TCS, and so on, all of which can be harmful to embryo development in vitro [19, 44, 48]. As we all know, air quality, plastic products, chemical pollutants, and other factors will affect oocytes and embryos, thus further affecting the rate of live birth. Therefore, it is very important to create an optimal environment for embryo culture to maintain the normal development of embryo, and obtain the stable pregnancy and live birth rate. Although exposure during fetal development is more likely to cause profound and permanent alterations than postnatal exposure, it is almost impossible to conduct such studies in human due to ethical issues. However, in IVF clinic, some evidences of EDCs affecting fertilization and embryo development may be found through epidemiological study and retrospective analysis.

From January 2013 to December 2019, 19 cases with indented oocyte ZP or waxy-like ZP were analyzed retrospectively in the reproductive center of Beijing Perfect Family Hospital. 18 of them had primary infertility more than 4 years, only one of them was secondary infertility, and the duration of infertility was 8 years. Most couples (79%) were classified as unexplained infertility, because the husbands had normal quality of semen and the wives with regular menstrual cycles was evaluated using routine parameters including complete hormonal profile and full cycle evaluation. Six patients had multiple failure of intrauterine insemination. 13 patients who underwent traditional IVF fertilization in the first cycle, 12 of them totally failed in fertilization and took early rescue intracytoplasmic sperm injection (ICSI). One of 13 patients had low rate of fertilization and early rescue ICSI was also performed. The remaining 6 patients' oocytes were fertilized directly by ICSI. According to our follow-up survey, most of female occupations have a history of hazardous EDCs contact. There are 6 patients engaged in clothing and footwear sales or production, 2 in fabric dyeing industry, 2 in cosmetic industry, 2 in decoration industry, and 3 in catering industry. Only four patients are unemployed and considered themselves without an obvious history of EDCs exposure.

In these cases, all oocytes present indented or waxy-like ZP, absence of resistance to ZP, and oolemma penetration during microinjection. On the day of oocyte retrieval, embryologists can observe the connection between oocytes and corona radiate becomes loose. Some patients' oocytes showed total or partial absence of PVS. Routine IVF is usually unable to fertilize, but ICSI has no effect on fertilization, suggesting that there are some problems in the sperm-ZP binding and penetration. The capacity to fertilize oocytes in abnormal ZP by ICSI, embryo development, and clinical outcomes seemed not to be compromised if these oocytes do not accompany by vacuoles in the cytoplasm, which usually have a poor outcome. 17 patients underwent 19 embryo transfer cycles, the pregnancy rate was 47% (9/19), and no miscarriage was seen. Seven patients delivered and nine healthy

babies were obtained, including 4 females and 5 males. There were 2 ongoing pregnancy (unpublished data).

The oocytes with indented ZP or waxy-like ZP mainly lead to fertilization failure in routine IVF process, which will absolutely cause poor clinical outcome if ICSI fertilization method is not used. Fortunately, ICSI can break through this barrier to achieve good IVF outcomes. As far as we know, most of patients' occupations had long-term exposure to EDCs. For example, patients engaged in clothing, footwear sales, and fabric dyeing industry may often be exposed to xylene, toluene, and other EDCs. Patients engaged in cosmetics industry may be primarily exposed to phthalates, parabens, triclosan, and VOCs. The decoration industry may be exposed to more EDCs such as formaldehyde, methane, and VOCs. The catering owners are mainly affected by cooking fumes, which usually contain acrolein. Our results suggest that EDCs may affect the ZP structure and characteristics of oocytes through blood circulation, resulting in the binding and penetration obstacle between sperm and ZP, and finally cause the problem of fertility. However, further studies are needed to link findings from epidemiological studies and experimental studies. The underlying molecular mechanisms of EDCs action on oocytes also are needed to further elucidate. However, it is very difficult to obtain convincing evidence in humans, because the experimental results are highly affected by various factors in the study design such as type of EDCs and their combined effects, length of exposure, dose, route of exposure, and potentially unhealthy participants.

9.2.2.1 BPA

Ehrlich et al. [29] reported a negative correlation between BPA levels and the rate of normally fertilized oocytes, with a decrease of 24 and 27%, respectively, for the highest versus the lowest quartile of urinary BPA. In addition, they found significantly reduced metaphase II oocyte count and the number of normally fertilizing oocytes, and also a suggestive relation between BPA urinary concentrations and reduced blastocyst formation, subsequently indicating that BPA might influence reproductive function in susceptible women seeking fertility treatment. As regarding to embryo development, a small study directed at the University of California in San Francisco detected no relations between serum BPA concentrations and embryo cleavage rate or fragmentation in 27 women undergoing IVF [91].

BPA can reduce and/or impair implantation [25, 33]. Some researchers considered that exposure to BPA might interfere implantation, because of the estrogenic properties of BPA, either by mismatch between the timing of blastocyst formation and the uterine receptivity window or by direct disruption of uterine receptivity to blastocyst implantation [89]. These endocrine activities of BPA have been demonstrated to result in harmful reproductive outcomes in animal models. Animal data have displayed that BPA primarily impairs female fertility by affecting the oviduct and uterus [92, 93]. Firstly, BPA influences oviduct morphology and gene expression, these changes may affect development and transport of the conceptus from the oviduct to the uterus. Secondly, BPA impacts uterine morphology and function and

thereby may lead to these changes over several generations via mechanisms that involve cell proliferation and receptivity. For example, BPA adversely influences ovarian steroidogenesis, modifies normal uterine morphology, and impairs uterine receptivity and implantation [19, 31, 94].

More than anything, exposure to BPA may specifically influence on the uterine adaptations during the preimplantation period in female. An early alteration of some gene expression such as *Hox10* affects the functional differentiation of the preimplantation uterus as part of an altered endocrine signal transduction pathway [34]. Furthermore, when there is an enough sample size, we suggest to explore the potential relationships of mixtures that include BPA with clinical pregnancy and live birth outcomes.

9.2.2.2 VOCs

Embryos are sensitive to the environment and VOCs are one of the vital factors affecting embryonic development [95]. Creating an optimal environment for embryo culture is crucial for ensuring embryo viability, and subsequently maintaining normal pregnancy outcome. It has been considered that air quality is essential for the success of IVF due to the presence of VOCs [44]. A study on optimizing laboratory conditions to decrease VOCs showed under optimum laboratory environmental conditions, the outcome of embryo quality was significantly improved. Blastocyst was featured by development of blastocoel in compacted embryo. Blastocyst formation rate raised around 18% after VOC reduction in the laboratory. Previous to remodel, the blastocyst formation rate was around 44.94%, then increased to around 62.83% [44]. Therefore, the implantation rate also increased from 31 to 42% as well as the live birth rate also raised from 23 to 31%. The number of good quality blastocysts was improved, that is, the cavity size of blastocoels was good and it appeared on time, increased cell number in the inner cell mass, and increased compactness of trophectoderm cell layer [44].

Air quality, in particular, is easy to overlook when pregnancy rates start to decline [96, 97]. Every embryologist must realize the importance of VOCs on the IVF outcomes [98]. So, more sensitive and optimized methods for controlling air pollution are justified to improve pregnancy outcomes especially in extended culture [43]. In addition, exposure to sporadic air contaminations may lead to sperm DNA damage and thereby raise the rates of male-mediated infertility, miscarriage, and other harmful reproductive outcomes [47, 99]. Air quality testing demonstrated improved air quality will significantly improve embryo implantation (32.4% versus 24.3%) and live birth (39.3% versus 31.8%), and it is indicated that improvements in IVF laboratory conditions and air quality had profound positive effects on laboratory measures and patient outcomes [100]. Many studies have detected that small amounts of VOCs can have damaging effects on pregnancy rates in the circulating air of an IVF laboratory [44]. High levels of VOCs (over 1 ppm) are directly toxic to embryos, which confirmed via human and mouse experiments. VOC levels around

0.5 ppm will typically allow for acceptable blastocyst development and reasonable pregnancy rates, but unfortunately, there are a high percentage of miscarriages [41].

9.2.2.3 Triclosan

Human studies exploring the effect of triclosan exposure on female reproductive health are limited. A recent Navigation Guide systematic review considered that TCS is "possibly toxic" to reproductive and developmental health [101]. In order to inquire whether high urinary TCS concentration is detrimentally correlated to early reproductive outcomes in women seeking fertility treatment, a study showed that a significant reduction of top quality embryo formation and implantation rate was displayed in women with urinary TCS concentration greater than or equal to the median level. Thus above study demonstrated that TCS exposure may exert harmful effects during early stages of human reproduction [102]. In addition, there was a prospective study designed to estimate the association between TCS exposure and early IVF outcomes for women undergoing IVF-ET. Not only top embryo formation rate and implantation rate seriously affected by higher TCS exposure, but a reduced trend of fertilization rate could be observed [102].

In addition, studies have suggested that preimplantation exposure to BPA and TCS can result in implantation failure in mouse models because of their ability to mimic estrogen in humans [54, 103, 104]. It has been considered that preimplantation exposure to the same amount of BPA or TCS on gestational day 2/3 is more potent to induce embryo implantation failure than exposure on gestational day 0/1 in mice [103]. Therefore, in mice, day 2–3 of gestation may be a sensitive window for BPA and TCS exposure. Exposure to these two EDCs during a sensitive window might result in implantation failure. In human beings, however, the sensitive window for these EDCs remain need further investigation [54, 104].

9.2.2.4 PFCs

High levels of PFCs in human FF have been related to subfertility [76, 82, 105]. In order to evaluate the effect of PFCs on oocytes quality and fertilization rate, a previous report analyzed the number of oocytes retrieved, oocytes quality, and the number of transferable embryos among women undergoing IVF. Results exhibited patients with FF PFC pollution had dramatically lower rate of fertilization and fewer number of transferable embryos. Moreover, there was a marked correlation between FF PFC levels and fertilization rate [81]. Overall, the aforementioned findings indicated harmful effect of PFC on oocytes fertilization capacity, blastocyst formation with the resultant decrease in the number of transferable embryos.

9.2.2.5 PCBs and OCPs

In spite of human data still largely inconclusive, PCBs have been related to detrimental reproductive health outcomes including decreased fecundability and increased risk of pregnancy loss [106]. Owing to their environmental ubiquity and persistence, PCBs can be detected in most of the general population [106]. Most of the studies concerns over the correlation between PCBs exposure and IVF outcome [106, 107]. First of all, they quantify OCPs concentrations in FF, then detect the use of serum as a biomarker of exposure to PCBs as compared to the potentially more biologically relevant biomarker of FF [108]. The current data showed that a significantly higher level of PCB congener (PCB 180) was found in the sera of patients who had no fertilization of oocytes [14], besides, rates of pregnancy were not markedly influenced by exposure to OCPs in IVF, but there was some evidence to suggest that OCPs decreased oocyte or embryo quality and implantation, while exposure to PCBs may be associated with failed embryo implantation. Another fact which may be relevant to human reproduction ability is that a negative association between fertilization and serum and FF *p,p*-DDE [14, 109].

As far as we known, nevertheless, there has only one study exploring the association between serum PCB concentrations and early pregnancy loss, the results showed serum PCB concentrations were related to failed implantation among women seeking IVF, the odds of failed implantation were doubled, and the odds of the live birth were decreased by 41% [110]. However, most studies reported previously shown there were no marked relationship was found between maternal PCBs and OCPs levels contamination and pregnancy outcomes. Based on the previous findings, it is still widely considered that chronic, low levels of maternal PCB pollution may do not affect a reproduction problem in humans and above evidence may reduce anxiety about harmful reproductive effects from chronic low-level PCB contamination [111, 112].

9.3 Implications for Human IVF Practice

It is very important to protect the gametes and embryos from exposing to harmful external factors. Although the embryo has a certain degree of adaptability to environmental change, it also produces physiological stress. Cell stress may also cause genetic imprinting and epigenetic changes in embryos, which may be inherited [113]. More evidence indicates that air quality in IVF laboratory plays an important role in IVF outcomes, because both particulate materials and VOCs are adverse to embryo development [41, 44]. Some researches show acetaldehyde, formaldehyde, and higher molecular weight aldehydes can affect the development or abortion of embryos, because VOCs can directly attach to embryonic DNA, and with the decrease of aldehydes levels, embryo development is improved [43, 114, 115]. A small amounts of VOCs in the air of an IVF laboratory can also have adverse effects

on pregnancy rate [116, 117]. Therefore, it is very important to establish an optimal embryo culture environment to ensure the viability of embryo, and thereby maintain the stability of pregnancy outcome.

A lot of reproductive centers are located in densely populated large city, whose air quality is worrying. IVF laboratory air quality faces many potential sources challenges, which include the construction materials; painting the laboratory; furniture; heating, air intake of HVAC (ventilation, and air conditioning); equipment and products used in the laboratory; incubator commissioning; plasticware; adhesives and sealants; smoking; packaging materials; cosmetics, perfume, hair and shoe covers; fibers from clothing; the cleaning, laundry and sanitizing products, and so on. The construction of IVF laboratory should minimize the generation of VOCs as much as possible. Despite the best effort to avoid their entry, VOCs will exist in the laboratory. It is essential for IVF laboratory to set up a primary, medium, and high efficiency particulate air filters system (HEPA) to achieve good air quality. However, HEPA filtration system can only filter particulate materials (including bacteria and mold spores) in the air, but cannot remove VOCs. Therefore, HVAC-based VOC filtration systems are designed to virtually eliminate VOCs in the air supplied to the laboratories. HEPA and HVAC-based VOC filtration systems, positive pressure can prevent airborne molecular, bacteria contaminants and reduce VOC levels. Because these microbes usually attach to the air particles. Positive pressure air flow in the laboratory coupled with the use of air purification systems may reduce the concentration of airborne particles and also reduces bacteria and other contaminants. To protect gametes and embryos from contracting VOCs in the laboratory, there are several approaches to achieve. Firstly, ventilation systems can be equipped with filters imbedded with activated carbon and potassium permanganate, which remove various hydrocarbons, such as benzene, formaldehyde, alcohols, and ketones. Another method for removing VOCs is ultraviolet photocatalytic oxidation (UVPCO). UVPCO uses the energy of UV lights absorbed by a semiconductor metal oxide to produce reactive species on the surface of the photocatalyst that then react with absorbed VOCs [118]. Furthermore, good indoor air quality should be obtained by using a portable or within-laboratory device such as the CODA (carbon activated air filtration) system and the Landson™ series. The CODA system consists of a gas inline filter to filter the gas entering the incubator and a tower to filter the air in IVF laboratory environment. Many studies have shown that the pregnancy and implantation rates have been improved after the CODA system is introduced into the IVF laboratory [44]. Khoudja et al. [113] reported that using air purification by the Landson™ series installation in the laboratory could significantly improve air quality in IVF laboratory. Embryo quality, the rate of pregnancy and implantation were also increased [41]. Finally, because VOC is oil-soluble, oil overlays of culture media can be used as an absorption tank to capture most VOCs. For keeping the acceptable rate of blastocyst development and pregnancy, the ideal VOC levels should be below 0.5 ppm, but it is better to be zero.

Both animal and human studies show that controlling laboratory contamination improves IVF outcomes [40, 95]. Although the available evidence in human on this topic is not very sufficient, it is hard to obtain such strong evidence owing to various

factors in study design and ethical issues. It is important for clinical embryologists to bear in mind that poor IVF outcomes may be due to such undesirable environmental exposure, and quality management and quality control in the IVF laboratory should be strengthened. In conclusion, all of these efforts help us maintain a clean, safe, and efficient IVF laboratory to achieve our ultimate goal—a live healthy baby.

References

1. Zhou Z, et al. Epidemiology of infertility in China: a population-based study. BJOG. 2018;125:432–41. https://doi.org/10.1111/1471-0528.14966.
2. Jain T, et al. 30 years of data: impact of the United States in vitro fertilization data registry on advancing fertility care. Fertil Steril. 2019;111:477–88. https://doi.org/10.1016/j.fertnstert.2018.11.015.
3. Fénichel P, Rougier C. Environmental factors and female reproduction. Encycl Endocr Dis. 2019;2:525–37. https://doi.org/10.1016/B978-0-12-801238-3.64950-4.
4. Ma Y, et al. Effects of environmental contaminants on fertility and reproductive health. J Environ Sci. 2019;77:210–7. https://doi.org/10.1016/j.jes.2018.07.015.
5. Sifakis S, Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. Environ Toxicol Pharmacol. 2017;51:56–70. https://doi.org/10.1016/j.etap.2017.02.024.
6. Patel S, Zhou C, Rattan S, Flaws JA. Effects of endocrine-disrupting chemicals on the ovary. Biol Reprod. 2015;93:20. https://doi.org/10.1095/biolreprod.115.130336.
7. Karwacka A, Zamkowska D, Radwan M, Jurewicz J. Exposure to modern, widespread environmental endocrine disrupting chemicals and their effect on the reproductive potential of women: an overview of current epidemiological evidence. Hum Fertil (Camb). 2019;22:2–25. https://doi.org/10.1080/14647273.2017.1358828.
8. Minguez-Alarcon L, Gaskins AJ. Female exposure to endocrine disrupting chemicals and fecundity: a review. Curr Opin Obstet Gynecol. 2017;29:202–11. https://doi.org/10.1097/GCO.0000000000000373.
9. Vander Borght M, Wyns C. Fertility and infertility: definition and epidemiology. Clin Biochem. 2018;62:2–10. https://doi.org/10.1016/j.clinbiochem.2018.03.012.
10. Kim YR, Pacella RE, Harden FA, White N, Toms L-ML. A systematic review: impact of endocrine disrupting chemicals exposure on fecundity as measured by time to pregnancy. Environ Res. 2019;171:119–33. https://doi.org/10.1016/j.envres.2018.12.065.
11. Brehm E, Flaws JA. Transgenerational effects of endocrine-disrupting chemicals on male and female reproduction. Endocrinology. 2019;160:1421–35. https://doi.org/10.1210/en.2019-00034.
12. Xin F, Susiarjo M, Bartolomei MS. Multigenerational and transgenerational effects of endocrine disrupting chemicals: a role for altered epigenetic regulation? Semin Cell Dev Biol. 2015;43:66–75. https://doi.org/10.1016/j.semcdb.2015.05.008.
13. Mahalingam S, et al. The effects of in utero bisphenol A exposure on ovarian follicle numbers and steroidogenesis in the F1 and F2 generations of mice. Reprod Toxicol. 2017;74:150–7. https://doi.org/10.1016/j.reprotox.2017.09.013.
14. Younglai EV, Foster WG, Hughes EG, Trim K, Jarrell JF. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing in vitro fertilization. Arch Environ Contam Toxicol. 2002;43:121–6. https://doi.org/10.1007/s00244-001-0048-8.
15. Petro EML, et al. Endocrine-disrupting chemicals in human follicular fluid impair in vitro oocyte developmental competence. Hum Reprod. 2012;27:1025–33. https://doi.org/10.1093/humrep/der448.

16. Varghese AC, Ly KD, Corbin C, Mendiola J, Agarwal A. Oocyte developmental competence and embryo development: impact of lifestyle and environmental risk factors. Reprod Biomed Online. 2011;22:410–20. https://doi.org/10.1016/j.rbmo.2010.11.009.
17. Jurema MW, Nogueira D. In vitro maturation of human oocytes for assisted reproduction. Fertil Steril. 2006;86:1277–91. https://doi.org/10.1016/j.fertnstert.2006.02.126.
18. Younglai EV, Holloway AC, Foster WG. Environmental and occupational factors affecting fertility and IVF success. Hum Reprod Update. 2005;11:43–57. https://doi.org/10.1093/humupd/dmh055.
19. Machtinger R, Orvieto R. Bisphenol a, oocyte maturation, implantation, and IVF outcome: review of animal and human data. Reprod Bio Med. 2014;29:404–10. https://doi.org/10.1016/j.rbmo.2014.06.013.
20. Krieg SA, Shahine LK, Lathi RB. Environmental exposure to endocrine-disrupting chemicals and miscarriage. Fertil Steril. 2016;106:941–7. https://doi.org/10.1016/j.fertnstert.2016.06.043.
21. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum Reprod Update. 2011;17:17–33. https://doi.org/10.1093/humupd/dmq032.
22. Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update. 2008;14:159–77. https://doi.org/10.1093/humupd/dmm040.
23. Gilchrist RB, Thompson JG. Oocyte maturation: emerging concepts and technologies to improve developmental potential in vitro. Theriogenology. 2007;67:6–15. https://doi.org/10.1016/j.theriogenology.2006.09.027.
24. Fadini R, et al. Oocyte in vitro maturation in normo-ovulatory women. Fertil Steril. 2013;99:1162–9. https://doi.org/10.1016/j.fertnstert.2013.01.138.
25. Pivonello C, et al. Bisphenol A: an emerging threat to female fertility. Reprod Biol Endocrinol. 2020;18:22. https://doi.org/10.1186/s12958-019-0558-8.
26. Peretz J, et al. Bisphenol A and reproductive health: update of experimental and human evidence, 2007–2013. Environ Health Perspect. 2014;122:775–86. https://doi.org/10.1289/ehp.1307728.
27. Huo X, et al. Bisphenol-A and female infertility: a possible role of gene-environment interactions. Int J Environ Res Public Health. 2015;12:11101–16. https://doi.org/10.3390/ijerph120911101.
28. Fujimoto VY, et al. Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during in vitro fertilization. Fertil Steril. 2011;95:1816–9. https://doi.org/10.1016/j.fertnstert.2010.11.008.
29. Ehrlich S, et al. Urinary bisphenol A concentrations and early reproductive health outcomes among women undergoing IVF. Hum Reprod. 2012;27:3583–92. https://doi.org/10.1093/humrep/des328.
30. Ferris J, Favetta LA, King WA, Bisphenol A. Exposure during oocyte maturation in vitro results in spindle abnormalities and chromosome misalignment in *Bos taurus*. Cytogenet Genome Res. 2015;145:50–8. https://doi.org/10.1159/000381321.
31. Lenie S, Cortvrindt R, Eichenlaub-Ritter U, Smitz J. Continuous exposure to bisphenol A during in vitro follicular development induces meiotic abnormalities. Mutat Res. 2008;651:71–81. https://doi.org/10.1016/j.mrgentox.2007.10.017.
32. Ferris J, Mahboubi K, MacLusky N, King WA, Favetta LA. BPA exposure during in vitro oocyte maturation results in dose-dependent alterations to embryo development rates, apoptosis rate, sex ratio and gene expression. Reprod Toxicol. 2016;59:128–38. https://doi.org/10.1016/j.reprotox.2015.12.002.
33. Tomza-Marciniak A, Stępkowska P, Kuba J, Pilarczyk B. Effect of bisphenol A on reproductive processes: a review ofin vitro, in vivoand epidemiological studies. J Appl Toxicol. 2018;38:51–80. https://doi.org/10.1002/jat.3480.

34. Caserta D, et al. Bisphenol a and the female reproductive tract: an overview of recent laboratory evidence and epidemiological studies. Reprod Biol Endocrinol. 2014;12:37. https://doi.org/10.1186/1477-7827-12-37.
35. Mahalingaiah S, Hauser R, Patterson DG, Woudneh M, Racowsky C. Bisphenol a is not detectable in media or selected contact materials used in IVF. Reprod Biomed Online. 2012;25:608–11. https://doi.org/10.1016/j.rbmo.2012.08.008.
36. Jain RB. Distributions of selected urinary metabolites of volatile organic compounds by age, gender, race/ethnicity, and smoking status in a representative sample of U.S. adults. Environ Toxicol Pharmacol. 2015;40:471–9. https://doi.org/10.1016/j.etap.2015.07.018.
37. Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E. Environmental and health impacts of air pollution: a review. Front Public Health. 2020;8:14. https://doi.org/10.3389/fpubh.2020.00014.
38. Catino A, et al. Breath analysis: a systematic review of volatile organic compounds (VOCs) in diagnostic and therapeutic management of pleural mesothelioma. Cancers (Basel). 2019;11:831. https://doi.org/10.3390/cancers11060831.
39. Toreyin ZN, Ghosh M, Goksel O, Goksel T, Godderis L. Exhaled breath analysis in diagnosis of malignant pleural mesothelioma: systematic review. Int J Environ Res Public Health. 2020;17:1110. https://doi.org/10.3390/ijerph17031110.
40. Esteves SC, Bento FC. Implementation of cleanroom technology in reproductive laboratories: the question is not why but how. Reprod Biomed Online. 2016;32:9–11. https://doi.org/10.1016/j.rbmo.2015.09.014.
41. Khoudja RY, Xu Y, Li T, Zhou C. Better IVF outcomes following improvements in laboratory air quality. J Assist Reprod Genet. 2012;30:69–76. https://doi.org/10.1007/s10815-012-9900-1.
42. Poletto K, de Lima Y, Approbato M. Effect of the air filtration system replacement on embryo quality in the assisted reproduction laboratory. Revista Brasileira de Ginecologia e Obstetrícia/RBGO Gynecol Obstetr. 2018;40:625–30. https://doi.org/10.1055/s-0038-1670715.
43. Mortimer D, et al. Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting. Reprod Biomed Online. 2018;36:658–74. https://doi.org/10.1016/j.rbmo.2018.02.005.
44. Agarwal N, et al. Volatile organic compounds and good laboratory practices in the in vitro fertilization laboratory: the important parameters for successful outcome in extended culture. J Assist Reprod Genet. 2017;34:999–1006. https://doi.org/10.1007/s10815-017-0947-x.
45. Mahalingaiah S. Is there a common mechanism underlying air pollution exposures and reproductive outcomes noted in epidemiologic and in vitro fertilization lab-based studies? Fertil Steril. 2018;109:68. https://doi.org/10.1016/j.fertnstert.2017.10.034.
46. Clark KL, Ganesan S, Keating AF. Impact of toxicant exposures on ovarian gap junctions. Reprod Toxicol. 2018;81:140–6. https://doi.org/10.1016/j.reprotox.2018.07.087.
47. Rubes J, et al. Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality. Hum Reprod. 2005;20:2776–83. https://doi.org/10.1093/humrep/dei122.
48. Dann AB, Hontela A. Triclosan: environmental exposure, toxicity and mechanisms of action. J Appl Toxicol. 2011;31:285–311. https://doi.org/10.1002/jat.1660.
49. Gore AC, et al. EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr Rev. 2015;36:E150. https://doi.org/10.1210/er.2015-1010.
50. Jurewicz J, et al. Triclosan exposure and ovarian reserve. Reprod Toxicol. 2019;89:168–72. https://doi.org/10.1016/j.reprotox.2019.07.086.
51. Zhang A, et al. Potential genetic damage to nematode offspring following exposure to triclosan during pregnancy. Mol Med Rep. 2017;16:1321–7. https://doi.org/10.3892/mmr.2017.6761.
52. Lange A, et al. Triclosan exposure and treatment outcomes in women undergoing in vitro fertilization. Fertil Steril. 2015;104:3. https://doi.org/10.1016/j.fertnstert.2015.07.264.
53. Arya S, Dwivedi AK, Alvarado L, Kupesic-Plavsic S. Exposure of U.S. population to endocrine disruptive chemicals (parabens, Benzophenone-3, Bisphenol-A and triclosan) and

their associations with female infertility. Environ Pollut. 2020;265:114763. https://doi.org/10.1016/j.envpol.2020.114763.
54. Yuan M, et al. Preimplantation exposure to Bisphenol A and Triclosan may lead to implantation failure in humans. Biomed Res Int. 2015;2015:1–9. https://doi.org/10.1155/2015/184845.
55. Johns LE, Cooper GS, Galizia A, Meeker JD. Exposure assessment issues in epidemiology studies of phthalates. Environ Int. 2015;85:27–39. https://doi.org/10.1016/j.envint.2015.08.005.
56. Lyche JL, et al. Reproductive and developmental toxicity of phthalates. J Toxicol Environ Health. 2009;12:225–49. https://doi.org/10.1080/10937400903094091.
57. Chin HB, et al. Association of urinary concentrations of phthalate metabolites and bisphenol A with early pregnancy endpoints. Environ Res. 2019;168:254–60. https://doi.org/10.1016/j.envres.2018.09.037.
58. Zhu YD, et al. Prenatal phthalate exposure and placental size and shape at birth: a birth cohort study. Environ Res. 2018;160:239–46. https://doi.org/10.1016/j.envres.2017.09.012.
59. Hauser R, et al. Urinary phthalate metabolite concentrations and reproductive outcomes among women undergoing in vitro fertilization: results from the EARTH study. Environ Health Perspect. 2016;124:831–9. https://doi.org/10.1289/ehp.1509760.
60. Machtinger R, et al. Urinary concentrations of biomarkers of phthalates and phthalate alternatives and IVF outcomes. Environ Int. 2018;111:23–31. https://doi.org/10.1016/j.envint.2017.11.011.
61. Kalo D, Roth Z. Low level of mono(2-ethylhexyl) phthalate reduces oocyte developmental competence in association with impaired gene expression. Toxicology. 2017;377:38–48. https://doi.org/10.1016/j.tox.2016.12.005.
62. Kalo D, et al. Mono(2-ethylhexyl) phthalate (MEHP) induces transcriptomic alterations in oocytes and their derived blastocysts. Toxicology. 2019;421:59–73. https://doi.org/10.1016/j.tox.2019.04.016.
63. Wang W, Craig ZR, Basavarajappa MS, Hafner KS, Flaws JA. Mono-(2-ethylhexyl) phthalate induces oxidative stress and inhibits growth of mouse ovarian antral follicles. Biol Reprod. 2012;87:152. https://doi.org/10.1095/biolreprod.112.102467.
64. Haman C, Dauchy X, Rosin C, Munoz JF. Occurrence, fate and behavior of parabens in aquatic environments: a review. Water Res. 2015;68:1–11. https://doi.org/10.1016/j.watres.2014.09.030.
65. Zhao X, et al. Occurrence, distribution, bioaccumulation, and ecological risk of bisphenol analogues, parabens and their metabolites in the Pearl River Estuary, South China. Ecotoxicol Environ Saf. 2019;180:43–52. https://doi.org/10.1016/j.ecoenv.2019.04.083.
66. Wang L, Kannan K. Alkyl protocatechuates as novel urinary biomarkers of exposure to p-hydroxybenzoic acid esters (parabens). Environ Int. 2013;59:27–32. https://doi.org/10.1016/j.envint.2013.05.001.
67. Cabaleiro N, de la Calle I, Bendicho C, Lavilla I. An overview of sample preparation for the determination of parabens in cosmetics. TrAC Trends Anal Chem. 2014;57:34–46. https://doi.org/10.1016/j.trac.2014.02.003.
68. Smith KW, et al. Urinary paraben concentrations and ovarian aging among women from a fertility center. Environ Health Perspect. 2013;121:1299–305. https://doi.org/10.1289/ehp.1205350.
69. Den Hond E, et al. Human exposure to endocrine disrupting chemicals and fertility: a case-control study in male subfertility patients. Environ Int. 2015;84:154–60. https://doi.org/10.1016/j.envint.2015.07.017.
70. Minguez-Alarcon L, et al. Urinary concentrations of bisphenol A, parabens and phthalate metabolite mixtures in relation to reproductive success among women undergoing in vitro fertilization. Environ Int. 2019;126:355–62. https://doi.org/10.1016/j.envint.2019.02.025.

71. Nowak K, Ratajczak-Wrona W, Gorska M, Jablonska E. Parabens and their effects on the endocrine system. Mol Cell Endocrinol. 2018;474:238–51. https://doi.org/10.1016/j.mce.2018.03.014.
72. Sabatini ME, et al. Urinary paraben concentrations and in vitro fertilization (IVF) outcomes. Fertil Steril. 2011;96:s154. https://doi.org/10.1016/j.fertnstert.2011.07.606.
73. Minguez-Alarcon L, et al. Urinary paraben concentrations and in vitro fertilization outcomes among women from a fertility clinic. Fertil Steril. 2016;105:714–21. https://doi.org/10.1016/j.fertnstert.2015.11.021.
74. Crawford NM, et al. Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility. Reprod Toxicol. 2017;69:53–9. https://doi.org/10.1016/j.reprotox.2017.01.006.
75. Raymer JH, et al. Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements. Reprod Toxicol. 2012;33:419–27. https://doi.org/10.1016/j.reprotox.2011.05.024.
76. McCoy JA, et al. Associations between perfluorinated alkyl acids in blood and ovarian follicular fluid and ovarian function in women undergoing assisted reproductive treatment. Sci Total Environ. 2017;605:9–17. https://doi.org/10.1016/j.scitotenv.2017.06.137.
77. López-Arellano M, et al. Effect of perfluorooctanoic acid in the generation of stress oxidative and cell death induction in mouse early oogenesis in vitro. Toxicol Lett. 2016;259:S231. https://doi.org/10.1016/j.toxlet.2016.07.555.
78. Hughes PM, et al. Peroxides in mineral oil used for in vitro fertilization: defining limits of standard quality control assays. J Assist Reprod Genet. 2010;27:87–92. https://doi.org/10.1007/s10815-009-9383-x.
79. Dominguez A, et al. Effect of perfluorooctane sulfonate on viability, maturation and gap junctional intercellular communication of porcine oocytes in vitro. Toxicol In Vitro. 2016;35:93–9. https://doi.org/10.1016/j.tiv.2016.05.011.
80. Dominguez A, et al. Effect of perfluorodecanoic acid on pig oocyte viability, intracellular calcium levels and gap junction intercellular communication during oocyte maturation in vitro. Toxicol In Vitro. 2019;58:224–9. https://doi.org/10.1016/j.tiv.2019.03.041.
81. Governini L, et al. The impact of environmental exposure to perfluorinated compounds on oocyte fertilization capacity. J Assist Reprod Genet. 2011;28:415–8. https://doi.org/10.1007/s10815-011-9548-2.
82. Kang Q, et al. Nontargeted identification of per- and polyfluoroalkyl substances in human follicular fluid and their blood-follicle transfer. Environ Int. 2020;139:105686. https://doi.org/10.1016/j.envint.2020.105686.
83. Toft G. Organochlorines and the effect on female reproductive system. Encycl Environ Health. 2015;4:778–84. https://doi.org/10.1016/B978-0-12-409548-9.09543-9.
84. Zhu Y, Huang B, Li QX, Wang J. Organochlorine pesticides in follicular fluid of women undergoing assisted reproductive technologies from central China. Environ Pollut. 2015;207:266–72. https://doi.org/10.1016/j.envpol.2015.09.030.
85. Pocar P, Brevini TA, Antonini S, Gandolfi F. Cellular and molecular mechanisms mediating the effect of polychlorinated biphenyls on oocyte in vitro maturation. Reprod Toxicol. 2006;22:242–9. https://doi.org/10.1016/j.reprotox.2006.04.023.
86. Pan W, et al. Selected persistent organic pollutants associated with the risk of primary ovarian insufficiency in women. Environ Int. 2019;129:51–8. https://doi.org/10.1016/j.envint.2019.05.023.
87. Campagna C, Ayotte P, Sirard MA, Bailey JL. An environmentally relevant mixture of organochlorines, their metabolites and effects on preimplantation development of porcine embryos. Reprod Toxicol. 2008;25:361–6.
88. Campagna C, et al. Effect of an environmentally relevant metabolized organochlorine mixture on porcine cumulus-oocyte complexes. Reprod Toxicol. 2007;23:145–52.

89. Go KJ. 'By the work, one knows the workman': the practice and profession of the embryologist and its translation to quality in the embryology laboratory. Reprod Biomed Online. 2015;31:449–58. https://doi.org/10.1016/j.rbmo.2015.07.006.
90. Ehrlich S, et al. Urinary Bisphenol A concentrations and implantation failure among women undergoingin vitro fertilization. Environ Health Perspect. 2012;120:978–83. https://doi.org/10.1289/ehp.1104307.
91. Bloom MS, et al. Serum unconjugated bisphenol A concentrations in men may influence embryo quality indicators during in vitro fertilization. Environ Toxicol Pharmacol. 2011;32:319–23. https://doi.org/10.1016/j.etap.2011.06.003.
92. Vigezzi L, et al. A deregulated expression of estrogen-target genes is associated with an altered response to estradiol in aged rats perinatally exposed to bisphenol A. Mol Cell Endocrinol. 2016;426:33–42. https://doi.org/10.1016/j.mce.2016.02.010.
93. Vigezzi L, et al. Developmental exposure to bisphenol A alters the differentiation and functional response of the adult rat uterus to estrogen treatment. Reprod Toxicol. 2015;52:83–92. https://doi.org/10.1016/j.reprotox.2015.01.011.
94. Ziv-Gal A, Flaws JA. Evidence for bisphenol A-induced female infertility: a review (2007–2016). Fertil Steril. 2016;106:827–56. https://doi.org/10.1016/j.fertnstert.2016.06.027.
95. Morbeck DE. Air quality in the assisted reproduction laboratory: a mini-review. J Assist Reprod Genet. 2015;32:1019–24. https://doi.org/10.1007/s10815-015-0535-x.
96. Franklin P, Tan M, Hemy N, Hall GL. Maternal exposure to indoor air pollution and birth outcomes. Int J Environ Res Public Health. 2019;16:1364. https://doi.org/10.3390/ijerph16081364.
97. Xue T, Zhang Q. Associating ambient exposure to fine particles and human fertility rates in China. Environ Pollut. 2018;235:497–504. https://doi.org/10.1016/j.envpol.2018.01.009.
98. Shah PS, Balkhair T. Air pollution and birth outcomes: a systematic review. Environ Int. 2011;37:498–516. https://doi.org/10.1016/j.envint.2010.10.009.
99. Caron-Beaudoin É, et al. Gestational exposure to volatile organic compounds (VOCs) in Northeastern British Columbia, Canada: a pilot study. Environ Int. 2018;110:131–8. https://doi.org/10.1016/j.envint.2017.10.022.
100. Heitmann RJ, et al. Live births achieved via IVF are increased by improvements in air quality and laboratory environment. Reprod Biomed Online. 2015;31:364–71. https://doi.org/10.1016/j.rbmo.2015.04.011.
101. Johnson PI, et al. Application of the Navigation Guide systematic review methodology to the evidence for developmental and reproductive toxicity of triclosan. Environ Int. 2016;93:716–28. https://doi.org/10.1016/j.envint.2016.03.009.
102. Hua R, et al. Urinary triclosan concentrations and early outcomes of in vitro fertilization-embryo transfer. Reproduction. 2017;153:319–25. https://doi.org/10.1530/REP-16-0501.
103. Crawford BR, Decatanzaro D. Disruption of blastocyst implantation by triclosan in mice: impacts of repeated and acute doses and combination with bisphenol-a. Reprod Toxicol. 2012;34:607–13. https://doi.org/10.1016/j.reprotox.2012.09.008.
104. Wang CF, Tian Y. Reproductive endocrine-disrupting effects of triclosan: population exposure, present evidence and potential mechanisms. Environ Pollut. 2015;206:195–201. https://doi.org/10.1016/j.envpol.2015.07.001.
105. Iwasaki Y, et al. Quantitative analysis of perfluorinated chemicals in media for in vitro fertilization and related samples. Chemosphere. 2012;88:445–9. https://doi.org/10.1016/j.chemosphere.2012.02.068.
106. Kadhel P, Monnier P, Boucoiran I, Chaillet N, Fraser WD. Organochlorine pollutants and female fertility: a systematic review focusing on in vitro fertilization studies. Reprod Sci. 2012;19:1246–59. https://doi.org/10.1177/1933719112446077.
107. Toft G, Thulstrup AM. Organochlorines and the effect on female reproductive system. Encycl Environ Health. 2011;1:275–82. https://doi.org/10.1016/B978-0-444-52272-6.00605-X.

108. Johnson PI, et al. Serum and follicular fluid concentrations of polybrominated diphenyl ethers and in-vitro fertilization outcome. Environ Int. 2012;45:9–14. https://doi.org/10.1016/j.envint.2012.04.004.
109. Toft G. Persistent organochlorine pollutants and human reproductive health. Dan Med J. 2014;61:B4967.
110. Meeker JD, et al. Serum concentrations of polychlorinated biphenyls in relation toin vitro fertilization outcomes. Environ Health Perspect. 2011;119:1010–6. https://doi.org/10.1289/ehp.1002922.
111. Jirsova S, Masata J, Jech L, Zvarova J. Effect of polychlorinated biphenyls (PCBs) and 1,1,1-trichloro-2,2,-bis (4-chlorophenyl)-ethane (DDT) in follicular fluid on the results of in vitro fertilization-embryo transfer (IVF-ET) programs. Fertil Steril. 2010;93:1831–6. https://doi.org/10.1016/j.fertnstert.2008.12.063.
112. Khanjani N, Sim MR. Maternal contamination with PCBs and reproductive outcomes in an Australian population. J Expo Sci Environ Epidemiol. 2006;17:191–5. https://doi.org/10.1038/sj.jes.7500495.
113. Wale PL, Gardner DK. The effects of chemical and physical factors on mammalian embryo culture and their importance for the practice of assisted human reproduction. Hum Reprod Update. 2016;22:2–22. https://doi.org/10.1093/humupd/dmv034.
114. Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: Methodologic issues in an emerging field. Basic Clin Pharmacol Toxicol. 2008;102:182–90. https://doi.org/10.1111/j.1742-7843.2007.00161.x.
115. Dadvand P, Rankin J, Rushton S, Pless-Mulloli T. Association between maternal exposure to ambient air pollution and congenital heart disease: a register-based spatiotemporal analysis. Am J Epidemiol. 2011;173:171–82. https://doi.org/10.1093/aje/kwq342.
116. William RB, et al. Control of air quality in an assisted reproductive technology laboratory. Fertil Steril. 1999;71:150–4. https://doi.org/10.1016/S0015-0282(98)00395-1.
117. Dickey RP, Wortham JWE, Potts A, Welch A. Effect of IVF laboratory air quality on pregnancy success. Fertil Steril. 2010;94:2. https://doi.org/10.1016/j.fertnstert.2010.07.605.
118. Hodgson AT, Destaillats H, Sullivan DP, Fisk WJ. Performance of ultraviolet photocatalytic oxidation for indoor air cleaning applications. Indoor Air. 2007;17:305–16. https://doi.org/10.1111/j.1600-0668.2007.00479.x.

Part IV
Effects of Environmental Factors on Reproductive Diseases

Chapter 10
Effects of Environmental Endocrine-Disrupting Chemicals on Female Reproductive Health

Qicai Liu

Abstract Environmental endocrine-disrupting chemicals (EDCs) are xenobiotic compounds that are frequently contacted in daily life. With the species and quantity of substances created and utilized by human beings significantly surpassing the self-purification capacity of nature, a large number of hazardous substances are enriched in the human body through the respiratory tract, digestive tract, and skin. Some of these compounds cause many problems endangering female reproductive health by simulating/antagonizing endogenous hormones or affecting the synthesis, metabolism, and bioavailability of endogenous hormones, including reproductive disorders, fetal birth defects, fetal developmental abnormalities, endocrine and metabolic disorders, and even gynecological malignancies. Therefore, the study of the relationship between environmental EDCs and female reproductive diseases and related mechanisms is of considerable significance to women, children health care, and improve the quality of the population.

Keywords EDCs · Reproductive disorders · CGRP · Hormone · Receptors · PCOS

10.1 The Mechanism of Environmental EDCs Affecting Female Reproductive Health

There are many kinds of EDCs with great structural differences, although the specific mechanism of their biological effects in the reproductive system is not yet clear. To sum up, they have two main effects: (1) mimic/antagonize endogenous hormones and (2) affect the synthesis, metabolism, and bioavailability of endogenous hormones. Endocrine disruptors can mimic endogenous the whole process of hormone metabolism and function, for example: hormone production, hormone

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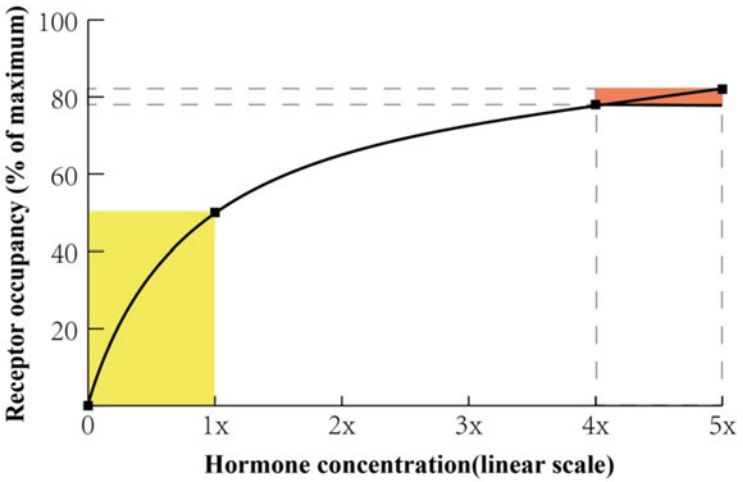

Fig. 10.1 Schematic of the spare receptor hypothesis

secretion, hormone transport, hormone metabolism, hormone binding, and its excretion [1]. Initially, EDCs were thought to act primarily by interfering with hormone binding by classical nuclear receptors. However, it is now well-established that EDCs have multiple modes of action that can interfere with transcription factors, non-steroid receptors (e.g., neurotransmitter receptors), orphan receptors (aryl hydrocarbon receptors), and enzymatic activities [2, 3].

EDCs can regulate hormonal function in vivo, especially for steroids. When EDCs bind to a specific hormone receptor, it can cause changes in hormone concentration and effect.

1. EDCs can mimic endogenous hormones, combining with hormone binding sites of receptors to form ligand–receptor complexes firstly and then binding DNA response elements, thereby showing the role of hormones and initiating a series of physiological and biochemical processes.
2. EDCs can also compete with endogenous hormones for receptors on target cells and reduce the adsorption of endogenous hormones by receptors, thereby enhancing the concentration and effect of endogenous hormones in other tissues.
3. In addition, EDCs can react with endogenous hormones directly or indirectly, such as by changing the function of endogenous hormones, affecting hormone synthesis, regulating the number of hormone receptors or their specific molecular affinity [4].

Physiological levels of endogenous hormones in healthy people are extremely low (e.g., estradiol on the order of pg/mL), but they have a strong affinity for the receptor (amplification of hormone action). At the same time, the receptor also follows the spare receptor hypothesis [5], whereby receptor occupancy gradually plateaus with increasing hormone concentration [6] (Fig. 10.1).

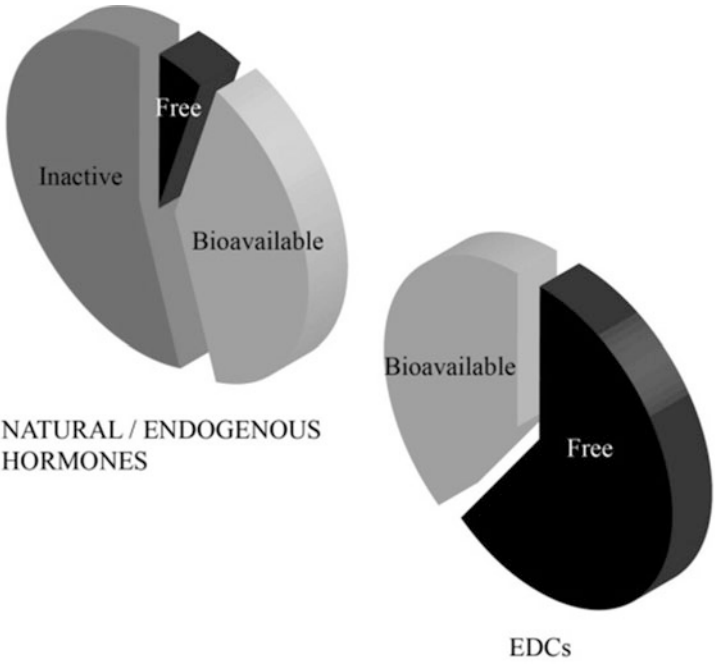

Fig. 10.2 EDCs regulate hormonal functions in vivo

Low concentrations: hormone concentration increase × (0–1×) causes receptor increase occupancy of approximately 50% (0–50%, shown in yellow box).

Higher doses: 4× to 5×, which causes receptor occupancy increase only approximately 4% (78–82%, shown in red box).

The release of hormones in the blood is usually pulse, and there is a positive and negative regulatory relationship between hormones and hormones [7, 8]. The frequency and amplitude of the pulse will regulate the biological response, and the secretion of hormones is also affected by the circadian rhythm [9, 10]. Importantly, the steroid hormones in the blood, acting as a buffering system, can be divided into three phases: free (active hormone), bioavailable (combine weakly to plasma proteins), and inactive (high affinity bound with proteins, for example, SHBG), which balance the circulating hormones. For EDCs, there may be almost all of them in the free phase. Thus, in the circulation, even low concentrations of EDCs may be physiology active and disrupt the balance of natural endogenous hormones (Fig. 10.2).

10.2 How EDCs Create Problems?

10.2.1 EDCs Interfere with Estrogens

During steroidogenesis, the P450scc enzyme (CYP11A1) converts cholesterol to pregnenolone. Steroidogenic enzymes convert pregnenolone to progesterone, androgen, and finally estrogen [4]. These include polychlorinated biphenyl compounds (PCBs), alkylphenols, phthalates (PAEs), diphenylalkanes/bisphenol compounds (BPs), organochlorine insecticides and herbicides, phytoestrogens (PEs) and fungal estrogens, and the heavy metal, lead, and nickel, all of which can interfere with the estrogen balance dependent on female reproduction.

PCBs, dihydroxy phenyl trichloroethylene (HPTE), alkylphenols, and PEs can activate estrogen receptors (ER) and exert estrogen-like effects. Tetrachlorodiphenylcyclodioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) organic polyhalogenated compounds, commonly referred to as dioxins because each molecule contains a dioxin backbone structure, inhibits estrogen receptor gene expression in mouse ovaries, uterus, and liver by decreasing estrogen receptor gene transcription, and this response may be mediated through the aryl hydrocarbon receptor [11, 12].

Estradiol secretion was significantly decreased in cultured luteal granulosa cells treated with TCDD, which suggests that TCDD may inhibit the endocrine function of human luteinized granulosa cells directly or indirectly by blocking mitogenic signaling through protein tyrosine kinase/microtubule-associated protein two kinases and protein kinase signaling [13]. The antifungal agent, methyl 2-benzimidazole carbamate (MBC), was first shown to exhibit similar phenomena in human ovarian granulosa cell cultures, and methyl-2-benzimidazole carbamate was reported to alter centrosome structure during mitosis in granulosa cells. One possible mechanism for this effect is impaired centrosome spindle microtubule dynamics, leading to metaphase arrest and abnormal chromosome morphology [14]. Zearalenone, the active product of the fungus, has also been associated with estrogenization, and pigs that had eaten “moldy corn” showed changes in mammary and reproductive tract function, and validation of these estrogenization performances by bioassays and receptor binding assays demonstrated that these natural products also have estrogenic activity [15].

10.2.2 EDCs Interfere with Androgens

Vinclozolin, procymidone, linuron, dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyl dichloroacetaldehyde (DDE) are all androgen receptor (AR) antagonists, which competitively inhibit androgen binding to its receptors and inhibit androgen-induced gene expression [16–19]. In animal experiments, reduced mammary duct branching and reduced lobular acinar development have

been observed in androgen receptor gene knockout female mice [20–22]. Moreover, increased susceptibility to carcinogenesis induced by DMBA (7,12-dimethylbenzanthracene, an experimental mammary carcinogen) was observed in ARKO mice [23], suggesting a close relationship between AR and carcinogenesis. In addition to endocrine disruptors with estrogenic/antiestrogenic activity, some EDCs such as pesticides also have antiandrogenic activity [24]. Vinclozolin is a potent antiandrogenic pesticide and also as a food contaminant. In vitro studies showed that administration of V drug in the uterus or early postnatal period could induce abnormal sexual differentiation and reproductive function in male rats [19, 25, 26]. Exposure to this antiandrogen compound was also associated with abnormal mammary gland development in female rats [27]. Dioxins and some PCBs can also induce the expression of cytochrome P450 enzymes (CYP1AI/CYP1A2) by binding to aryl hydrocarbon receptor (AhR), accelerate the degradation of estrogen in vivo, produce the antiandrogenic effect, and affect the underlying cellular processes such as cell growth, differentiation, and programmed cell death.

10.2.3 EDCs Interfere with Thyroxine

Normal brain development requires the maintenance of thyroid hormones, besides, thyroid hormones are essential to regulate metabolism and maintain normal physiology [28], while EDCs exposed in life can induce changes in thyroid structure and function at the HPT axis (hypothalamic–pituitary–thyroid) (Fig. 10.3), including the synthesis, release, transport, and metabolism of thyroid hormones or the effects of thyroid hormones on target tissues [29–31]. HPT function is regulated primarily through two complex pathways, and one is that the target organ or tissue can regulate itself sensitivity to thyroid hormone by altering the expression of receptor or metabolizing enzyme [32]. On the other hand is that EDCs can interfere with the effects of thyroid hormones in tissues and cells regulated by the thyroid, hypothalamic, pituitary, or thyroid hormones in complex ways, implying that the ability of EDCs to interfere. The function of thyroid hormone should be evaluated comprehensively, not only depending on the level of thyroid hormone in the blood [33].

It is a highly complex process to regulate the transfer of thyroid hormone to target tissues, including the synthesis of thyroid, the transportation through blood, the selective uptake into tissues and cells, and the metabolism of the action site.

1. TRH can be produced by small cell neurons in the paraventricular nucleus (PVN) of the hypothalamus, only a part of which control the release of TSH (pituitary stimulation) from the pituitary gland. These neurons are jointly controlled by the neurotransmitter and the thyroid hormone negative feedback regulation serum T4, in which T4 is transformed from tanycytes to T3, which is transmitted to TRH neurons through the cell transporter MCT8. Negative feedback itself is selectively mediated by the thrb2 receptor. It stimulates the synthesis and release of TSH in pituitary through the action of membrane receptor, which signals through protein

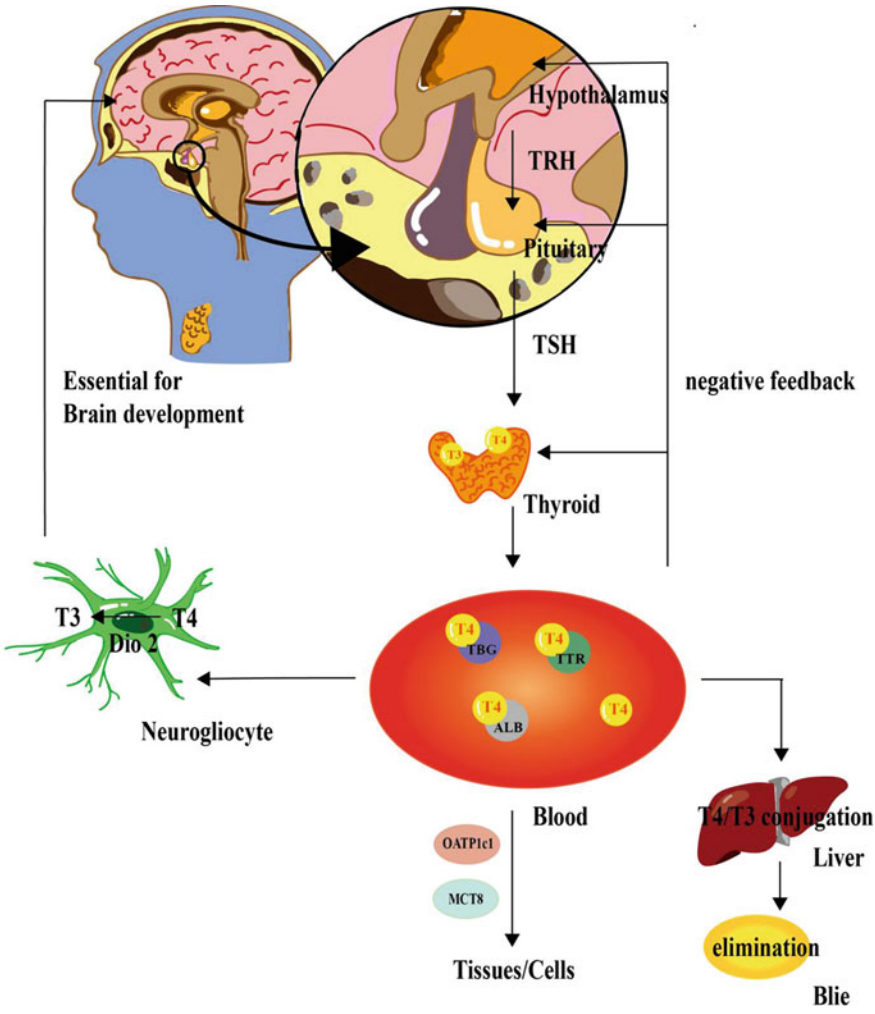

Fig. 10.3 Hypothalamic–pituitary–thyroid (HPT) axis

kinase C. TSH stimulates thyroid cells through camp and increases the synthesis and release of thyroid hormones.

2. The release of thyroid hormone requires the endocytosis of thyroglobulin in the colloid through the vesicle transport of thyroid cells. In this process, the thyroglobulin residues are coupled and removed from the protein skeleton before being released.
3. Thyroid hormones are carried by binding proteins (the so-called distribution proteins). About 75% of T4 is carried by TBG (T4-binding protein), additional 25% by TTR (transthyretin), a small amount by albumin, and only about 0.01% remaining “free state” (unbound).

4. T4 in serum enters tissues and cells through selective transporters. The transmission of bioactive T3 is very complex, especially in the nervous system. T4 was absorbed by glial cells and transformed into T3 by deiodinase-2 (DIO 2). T3 is then actively transported to neurons and acts on ThR α or β.
5. The half-life of T4 in human serum is 7–10 days, while that in rodents is 24 h. T4 is controlled by the liver, which expresses enzymes (glucuronidase or thiotransferase) that modify T4 and T3, thus eliminating them in bile [4].

Chemicals that have a direct effect on the thyroid gland, perchlorate, chlorate, nitrate, thiocyanate, and other complex anions, act directly on the thyroid gland to interfere with sodium-iodide symporter function and affect iodine uptake, thereby interfering with thyroid function, especially when iodide intake is low to insufficient [34]. A recent study showed that the level of perchlorate in pregnant women with critical thyroid function was negatively correlated with the cognitive function of their offspring [35].

Dioxins, phthalates, bisphenol A, brominated flame retardants, malathion, and perfluorinated chemicals, all interfere with the balance of thyroid hormones in the human body [36, 37]. Organochlorine compounds, especially PCBs, are significantly associated with FSH and FT3 in humans. The marked decrease in serum TT4, TT3, and thyrotropin-releasing hormone levels in rats exposed to PCBl53 may be related to its association with the polycyclic aromatic hydrocarbon receptor and induction of increased hepatic production of uridine diphosphate glucuronosyltransferase [31]. PCBs have also been shown to affect the activity of thyroid hormone deiodinase, and the activity of hepatic deiodinase-1, which catalyzes the conversion of T4 to T3, was inhibited in rats exposed to PCBl53 [38]. It is now known that PCBs can affect thyroid hormone levels in blood and tissues through at least a variety of independent but interacting pathways, such as by altering thyroid tissue structure, affecting thyroid hormone metabolism, and competing for carriers [39]. Although not all studies have reported the same effects of PCBs on serum thyroid hormone levels [36, 40], subtle changes in the thyroid gland may have significant short- and long-term effects on development, especially during sensitive developmental periods [41–43]. Neurodevelopment is particularly sensitive in pregnant women and their fetuses, preterm infants, and infants, susceptible to permanent effects [41, 43], while older children and adolescents may primarily exhibit adverse effects related to growth and reproductive development. The prevalence of thyroid peroxidase antibody was significantly higher in reproductive age women exposed to PCBs than in those not exposed, and the prevalence of TSH receptor antibody was also significantly higher.

Physiological thyroid hormone levels play a key role in maintaining the stability of the hypothalamic–pituitary–ovarian axis. Thyroxine can directly participate in and affect the process of estrogen metabolism and also inhibit the differentiation and proliferation of ovarian endocrine cells. Thyroxine regulates ovarian function through pituitary secretion of gonadotropins (Gn), including follicle stimulating hormone (FSH) and luteinizing hormone (LH). A small amount of thyroxine promotes LH secretion, while a large amount of thyroxine inhibits Gn secretion

[44]. Thyroid hormones can directly reduce ovarian responsiveness to GN from pituitary [45] and modulate circulating estrogen activity by increasing testosterone–estrogen binding globulin [44]. In addition, thyroid hormones can directly affect the maturation of human oocytes [46], which express thyroxine receptors (TRα1, TRβ1) and TSH receptors, among others. Therefore, EDCs indirectly or directly affect female reproductive health by interfering with thyroid hormone levels or even through thyroid hormone-like effects.

10.2.4 EDCs Interfere with Other Endocrine Hormones

T-octane phenol, nonanol, pentachlorophenol, DDT, and hexachlorocyclohexane can bind to progesterone receptors and exert antiprogestational effects. Through influence the activity, transcription, and expression of steroidogenic enzymes, EDCs lead to changes in steroid hormone levels and biological effects; by competitively binding to serum albumin and sex hormone-binding protein, EDCs reduce the adsorption of endogenous hormones to target cells, thereby enhancing the action of endogenous hormones; and by affecting the activities of hepatic microsomal metabolic enzymes, cytochrome P450 enzymes, UDP-glucuronosyltransferase and other enzymes, EDCs lead to changes in steroid hormone levels [47, 48].

10.2.5 Combined Effect of EDCs on Nervous System, Endocrine System, and Immune System

EDCs affect the nervous system in two ways: first, they act on the neuroendocrine system to affect the release of hormones and the effects of target organs; second, they act directly on the nervous system to cause changes in behavior and spirit. The mechanisms by which environmental disruptors induce or accelerate the autoimmune disease process may be: (1) to alter gene expression in cells involved in the immune response; (2) to drive the release of autoimmune cells to the periphery; (3) to alter certain molecules of themselves; and (4) to impede the secretion of thymosin, thereby inhibiting the maturation of T cells, which may attack self cells and trigger autoimmune diseases [49].

Calcitonin gene-related peptide (CGRP) is a small 37-amino acid neuropeptide encoded by selective cleavage of the calcitonin gene. CGRP is well-known to researchers due to its potent vasodilator function, but CGRP has been less studied in the reproductive system. CGRP has a negative immunomodulatory function, and there are two peptides with similar biological activities, αCGRP and βCGRP, which act as mediators between neural, immune, and endocrine, maintaining the homeostatic balance of the internal environment [50]. Studies have found abnormal CGRP

secretion, loss of inhibitory effect on inflammatory cells resulting in occlusive vasculitis and perineuritis (Fig. 10.4).

CGRP functions by regulating cytokines, such as inhibiting the secretion of TNF-a by macrophages, decreasing the production of IFN-γ by T cells, and increasing the anti-inflammatory cytokine IL-10 [51]. Endocrine hormone disorder is a significant cause of embryo implantation failure, and abnormal neuropeptides, etc. also directly affect the rate of mouse embryo implantation [52]. Thus, the theory of "Endometrial Receptivity for Embryo Implantation Programmed Opening" has become a hot topic in the field of reproductive medicine [53, 54].

Supplementary Description: Hypothesis of Endometrial Decidualization Process

We previously observed that endometrial CGRP is upregulated during embryo implantation in a murine model. Considering the well-established role of CGRP in electrolyte transport and water reabsorption [55, 56], we speculate that CGRP upregulation is associated with the disappearance of fluid in the uterus and the closure of the uterine cavity normally observed. Moreover, CGRP has been shown to contribute to prostaglandin release by both mechanical force and serine protease activation [57]. Therefore, we propose the hypothesis that CGRP induces calcium currents, conformational changes in the nuclear pore $Nup_{62\text{-}69}$, and increased LIF entry, ultimately leading to the production and release of prostaglandins necessary for the decidualization process and embryo implantation (Fig. 10.5).

10.3 The Harm of Environmental Endocrine Disruptors to Female Reproductive System

Normal development and function of the female reproductive system depend on hormone levels and balance. Endocrine disorders will lead to menstrual disorders, infertility, endometriosis, polycystic ovary syndrome, and other diseases. These abnormal diseases are caused by the abnormal regulation of estrogen, androgen, and thyroid hormone levels, in which EDCs play an essential role. Research evidence suggests that the potential effects of EDCs are derived from diethylstilbestrol (DES).

Diethylstilbestrol is a synthetic non-steroidal estrogen, which can produce all the same pharmacological and therapeutic effects as natural estradiol. It is mainly used for functional bleeding and amenorrhea caused by hypoestrogenism and hormone imbalance. It can also be used to improve the sensitivity of myometrium to oxytocin before induction of stillbirth. Long-term stimulation of the uterus by DES is a high risk factor for clear cell carcinoma of the vagina [58, 59]. DES has also been associated with menstrual irregularities, uterine malformations, infertility, miscarriage, preterm labor, and breast cancer [59–61].

Studies have found differences in pregnancy-related physiological changes between human and rodent experimental observations, For example, the

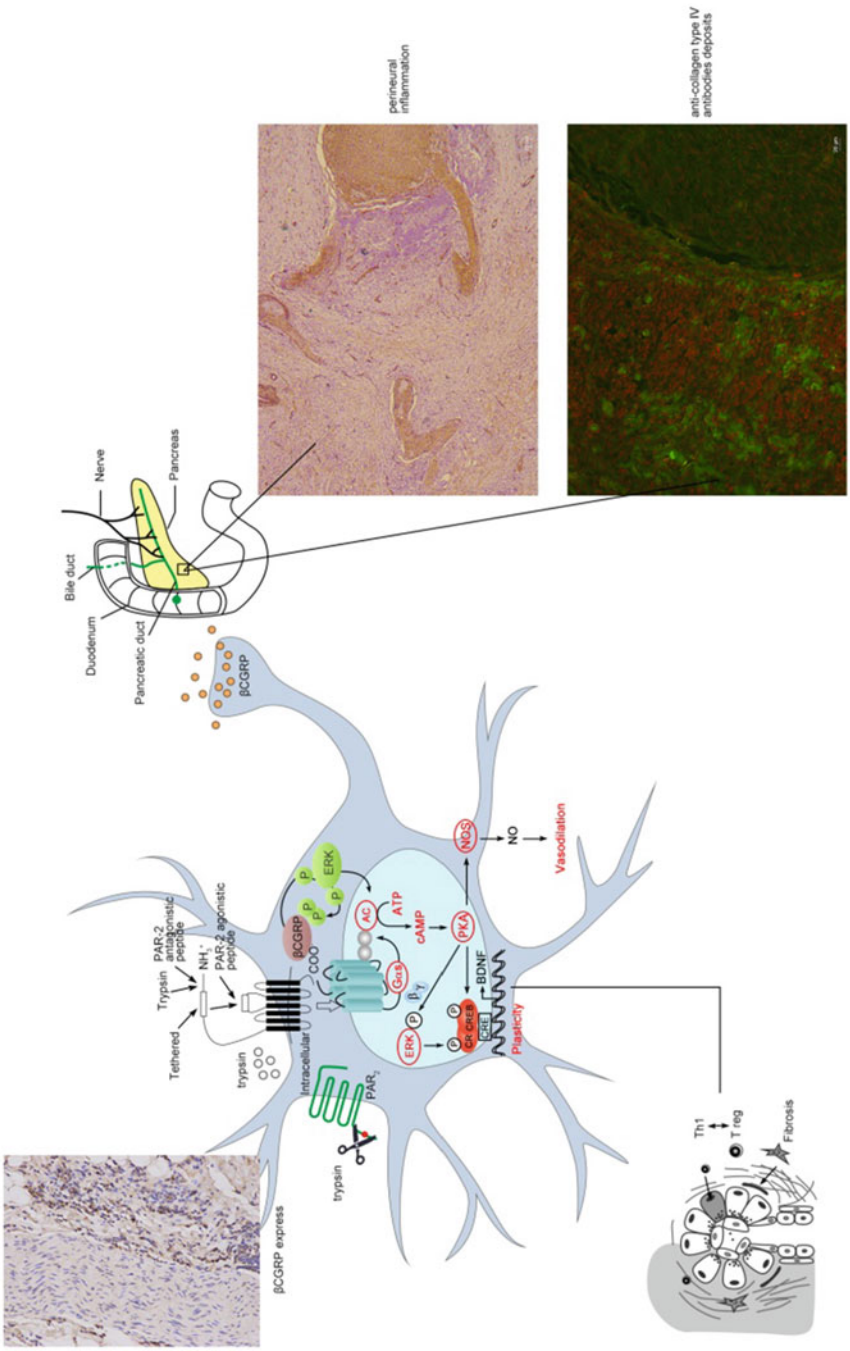

Fig. 10.4 Correlation between neuropeptide CGRP and neurovasculitis

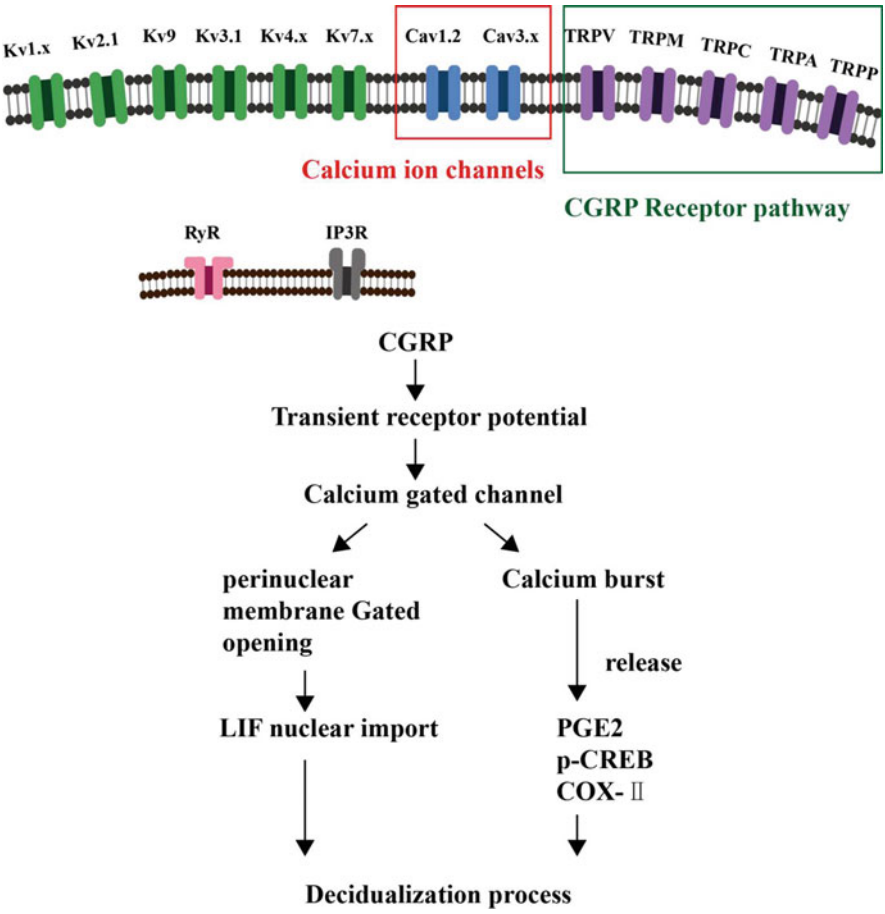

Fig. 10.5 Hypothesis diagram of endometrial decidualization process

involvement and hormone control of the corpus luteum, the organs involved in progesterone and estrogen secretion, the specific estrogen produced, and the blood estrogen level obtained in the mother and fetus. In particular, the level of estrogen obtained during human pregnancy is significantly higher than that obtained in mice, and the adverse endocrine-disrupting effect of low-dose bisphenol A or other exogenous estrogens on human pregnancy seems to be dwarfed [62], which reminds us that EDCs cannot be studied only at the laboratory level. Many EDCs have a mechanism of action similar to that of DES, as an exogenous estrogen. However, the similarity between DES and EDCs could not be determined in the present study, because the duration of action of DES was limited, while EDCs played a role in the whole life process.

10.3.1 Adverse Pregnancy Outcomes and Pregnancy Complications

EDCs can cause chromosomal aberration in oocytes, immaturity of oocytes, and lead to pregnancy failure; affect the embryo development and implantation, resulting in early pregnancy loss and unnoticed abortion; embryo dysplasia and abortion; interfere with the expression of key genes of embryonic development, leading to abnormal embryonic development and differentiation. Human studies have revealed the relationship between bisphenol A (BPA) and in vitro fertilization (IVF) hormone levels. In one report, higher BPA levels were associated with lower peak serum estradiol levels in women undergoing in vitro fertilization prior to oocyte retrieval [63–65]. In addition, bisphenol A exposure was associated with increased testosterone, estradiol, and pregnenolone levels in girls with precocious puberty [66, 67].

BPA exposure decreased the number of antral follicles and oocytes, resulting in a lower probability of conception [68]. PCBs block oocyte maturation, decrease fertilization rate, and increase polyspermy [69] and can cause stillbirth and fetal growth retardation. Cadmium has obvious toxic effect on ovary and oocytes and causes chromosome aberration in mouse and hamster oocytes. Organotin can disturb calcium homeostasis and change cytoskeleton to block embryo development at the stage of morula or blastocyst, resulting in early pregnancy abortion and fetal malformation [70]. The pregnancy rate of female workers exposed to PAEs (plasticizer) decreased and abortion rate increased, and the incidence of anemia during pregnancy, hyperemesis gravidarum, and pregnancy-induced hypertension syndrome increased. The incidence of spontaneous abortion, premature delivery, and stillbirth increased in female workers exposed to lead, mercury, and manganese. Exposure to lead and mercury may cause intrauterine growth retardation and low birth weight. Lead can cause central nervous system malformation and even stillbirth in young rats in animal experiments.

10.3.2 Endometriosis (EMs)

EMs have a serious impact on the physical and mental health and quality of life of many women. EDCs may be involved in the development of diseases. Melissa et al. estimated the human evidence for endocrine disruptors and endometriosis, limited quantitative studies to individual chemical concentrations in women, including controls in unaffected women, and used multivariate analysis techniques to support environmental causes of endometriosis, including metals/trace elements, dioxins and other persistent organic pollutants, as well as non persistent organic pollutants, sexual chemicals, such as benzophenone and phthalate [71]. Cadmium exposure significantly increased the diagnosis of endometriosis, and urinary chromium and copper levels increased EMS prevalence by two times or more [72, 73].

Studies on the correlation between EDCs and EMs have been available as early as the 1990s, with dioxins being the most studied. A direct relationship between dioxin and the onset of EMS was first reported by Rier in 1993. The incidence rate of EMs in Ganges River monkeys after 4 years of exposure to dioxin was significantly higher than that in control group 10 years later, and the severity of illness was positively correlated with the exposure dose. EMs occur in all macaques that have been exposed to dioxins for 17 years [74]. The incidence and severity of EMs in Belgian women are among the highest in the world, consistent with the severity of dioxin pollution in the country's environment. They have caused extensive research on the relationship between dioxins and the incidence of EMs worldwide. In the EMs mouse model, dioxin promotes the growth of ectopic lesions in a dose-dependent manner: the number of lesions increased by two times compared with the control group when the endometrium treated with dioxin was transplanted into the abdominal cavity of nude mice, and it was considered that interference with the expression of matrix metalloproteinase mediated by progesterone enhanced the secretion of cytokines by endometrial immune cells.

Research on the relationship between dioxins and EMs will continue [72, 75]. Epidemiological studies have shown that the serum levels of DEHP, MEHP, and PCBs in patients with EMs are significantly higher than those in healthy women [4], and in vitro experiments have shown that MEHP contributes to the development of EMs by increasing the activity of endometrial stromal cells [76]. As an estrogen dependent disease, estrogen exposure in uterus may be related to endometriosis, while diethylstilbestrol exposure may be related to the occurrence of endometriosis [77, 78].

10.3.3 Polycystic Ovary Syndrome (PCOS)

PCOS is one of the most common gynecological endocrine diseases, characterized by clinical or biochemical manifestations of androgen excess, persistent anovulation, polycystic ovarian changes, often accompanied by insulin resistance and obesity, and heterogeneous diseases whose etiology is still not elucidated. Current studies suggest that it may be due to the interaction between specific genetic genes and environmental factors. EDCs, especially BPA, a group of widely distributed pollutants, are possible environmental factors in the pathogenesis of PCOS. From in vitro and animal studies it was demonstrated that endocrine disruptors induce reproductive and metabolic abnormalities similar to the features of PCOS [79]. In a survey, researchers measured and compared the bisphenol A levels of patients with PCOS, reproductive health women, and a group of healthy men. The results showed that the bisphenol A levels of normal men and women with PCOS were significantly higher than those of normal women. In addition, BSA levels in all subjects were positively correlated with serum total testosterone and free testosterone, indicating that this chemical may play a role in the pathophysiology of PCOS. It is assumed that BPA directly stimulates the secretion of androgen by ovarian membrane cells by

upregulating the expression of 17 β hydroxylase P450c17, cholesterol side chain lyase P450scc, and steroidogenic acute regulatory protein (StAR) [80]. Moreover, BPA can indirectly increase androgen by changing androgen metabolism in liver through two mechanisms. The first mechanism involves the interaction between BPA and sex hormone binding globulin, which may replace androgen and interfere with androgen/estrogen balance, leading to an increase in circulating androgen levels [81]. The second mechanism may involve BPA downregulation of androgen metabolism/hydroxylation by reducing the activity of testosterone 2A hydroxylase and testosterone 6B hydroxylase, which are the enzymes responsible for this process [82]. On the other hand, androgen itself affects the metabolism of BPA by reducing the activity of UGT. This enzyme catalyzes the glucuronization of bisphenol A through liver microsomes [83]. It has been proved that the structural binding of BPA and its more effective metabolite 4-methyl-2,4-bis (4-hydroxyphenyl)-1-pentene (MBP) with androgen receptor (AR) and progesterone receptor (PR) may inhibit the binding of endogenous AR and PR ligands, leading to target tissue dysfunction [84].

As this study is still in its infancy, further studies are needed to understand the mechanism of action of EDCs on PCOS, as well as the critical period of exposure, to determine the molecular basis of the observed reproductive and metabolic alterations caused by BPA and to determine how these effects affect offspring through epigenetic mechanisms. Future studies should also aim to extrapolate data obtained from animal studies to humans with major hurdles to overcome species differences and determine whether other EDCs besides BPA need to be considered [85].

10.3.4 Premature Ovarian Failure (POF)

More and more experimental animal models and epidemiological data show that exposure to a series of reproductive toxic environmental chemicals (RTECS) can lead to premature menopause, even premature ovarian failure. More and more women around the world suffer from early menopause and POF (about 0.4% under the age of 35 and 1% under the age of 40) [86], and genetic, autoimmune, infectious, metabolic, and medical or environmental factors seem to play a role [87–92]. It is closely related to ovarian reserve failure and mainly affects the early and late establishment of ovarian reserve. The loss of human reproductive ability is mainly caused by the continuous failure of follicles. The insufficient number of germ cells formed in the prenatal period leads to a lack of follicular pool (decreased ovarian reserve) at birth and/or adolescence and accelerated depletion of established ovarian reserve. The increased activation of primary follicles (PFS) and follicular atresia (increased failure rate) are the key factors leading to premature ovarian failure. Recently, several retrospective studies have explored the effects of exposure to EDCs on ovarian development. Maintaining the normal development of follicle/oocyte and the normal secretion of steroid hormones are the key factors to ensure

ovarian function. It is worth noting that many other environmental chemicals can induce ovarian toxicity, independent of hormone signaling [93].

Combined exposure to phthalate and 4-ethylcyclohexene dioxide can accelerate follicular failure and lead to premature ovarian failure in rats. Among the follicular toxic substances, polycyclic aromatic hydrocarbons (PAH), 4-vinyl-1-cyclohexene (VCH) and its metabolite 4-vinyl-1-cyclohexene diepoxide (VCD) have been the most studied. PAH-like compounds can be produced when minerals burn, but because of the large number of smokers, cigarettes become a major source of exposure to such compounds, including dioxins (TCDD, PCDDs, PBDDs), PCBs, PBBs, etc. VCH is produced in the production of rubber tires, burning resistance agents, pesticides, plasticizers, antioxidants. PAHs can activate AhR and induce the expression of Bax in oocytes, which in turn leads to the apoptosis of cells [94]. VCH and VCD damaged primordial and primary follicles of female rats and mice, which was considered to be related to the increased expression of Bax and the activity of caspase-2 and caspase-3, thereby promoting apoptosis. Takai et al. found that VCD disrupted primordial and primary follicles in BAX knockout mice, and there was less destruction of primary follicles in caspase-2 and caspase-3 knockout mice than in wild-type mice [95]. In 2012, Hunter and colleagues investigated the role of circulating levels of BPA in humans in meiotic initiation and primordial follicle formation during the second and third trimesters of pregnancy. They found that (1) oocytes exposed to BPA showed a higher recombination rate in the pachytene phase and (2) exposure to BPA reduced the percentage of secondary and sinusoidal follicles, whereas the frequency of multioocyte follicles (MOFs) was significantly increased in the offspring [96]. Adult female rats treated with BPA (0.001 and 0.1 mg/kg/day) promoted follicular atresia and luteal regression by inducing apoptosis of caspase-3 related cells [97]. In addition, a low dose (100 μg/mL) of BPA also increased Bax expression, inhibited the transcription and expression of Bcl-2, and induced apoptosis. At the same time, the level of transition-related protein 53 (Trp53) was increased, cells were arrested in G2 to M phase, DNA was damaged, the viability of ovarian granulosa cells was inhibited, and follicular atresia was promoted [98, 99]. In utero exposure to low doses (50 mg/kg/day) of BPA during critical ovarian developmental windows has also been found to interfere with early ovarian development and animal estrus, and BPA exposure has been shown to affect steroidogenesis and reduce fertility with aging in a variety of animal models [100]. There are other environmental toxic exposures that significantly affect reproductive health (Table 10.1).

10.3.5 Gynecologic Tumor

In recent years, the incidence of hormone-dependent organ tumors has been increasing [106], especially in female hormone-sensitive organs such as ovary, uterus, and breast. EDCs participate in the occurrence and development of tumors through multiple pathways. The complexity of the causes of tumors and the diversity of the effects of EDCs make it still difficult to identify the direct relationship between

Table 10.1 Development of primordial germ cell damage by environmental toxic exposure

References	EDC types	Exposure (dpc)	Exposure	Affected processes
La Sala et al. [101] (CD-1 mice)	15–30 mg/kg; 10–20 nM lindane 20 ng/g BPA	11.5	Gavage	Increase apoptosis
Lawson et al. [102] (C57BL/6J mice)	20 ng/g BPA	11.5–12.5	Oral	Downregulation of mitotic cell-cycle genes
Zhang et al. [103] (CD-1 mice)	40–160 μg/kg BPA	0.5–12.5	Oral	Decreased expression of germ cell specific genes; DNA methylation
Li et al. [104] (CD-1 mice)	40 μg/kg DEHP	0.5–18.5	Oral	DNA methylation (female and male transgenerational transmitted)
Holm et al. [105] (C57BL/6J mice)	50, 150 mg/kg paracetamol	7–13.5	Gavage	Decreased proliferation and/or Increased apoptosis

EDCs and the occurrence and development of tumors. Experimental studies have shown that early exposure to estrogen-like chemicals (exogenous estrogens, such as BPA), even at low dose levels, can increase the susceptibility of rodents to chemically induced breast cancer, possibly by changing breast development and/or destroying the ratio of proliferating/apoptotic cells [107–109]. Warner et al investigated 981 women (aged 40 years at the time) affected by a TCDD leak in Italy in 1976, 15 of whom had breast cancer with a serum TCDD concentration of 13–1960 ppt. And it was found that when the degree of TCDD in serum increased 10 times, the risk of breast cancer increased 2.1 times [110]. However, the opposite results were also reported, suggesting that TCDD may be a chemoprotective agent against breast cancer and that TCDD inhibits the proliferation of AhR (aryl hydrocarbon receptor) in tumor cells and infiltration of normal tissues [111]. A review of the existing literature shows that soy food consumption or exposure to genistein (g) during female childhood and adolescence, as well as before the onset of puberty, can reduce the risk of breast cancer in the future. Rodents exposed to G in utero, during lactation or before puberty, even at low doses, can reduce the incidence and diversity of breast cancer induced by carcinogens by promoting breast differentiation, changing cell proliferation, apoptosis, and upregulating tumor suppressor genes. This reduces the incidence and diversity of carcinogen-induced mammary tumors. In the study of the association between PCBs, organochlorine pesticides, and breast cancer, which may be limited by methodology, sample size, there are also different findings. In addition to an increased incidence of breast cancer, DES use during pregnancy was associated with an increased incidence of vaginal clear cell carcinoma in female offspring. Through a combination of experimental and epidemiological approaches, Kim et al. found that PAEs can promote the development of uterine fibroids by promoting the proliferation of myometrial smooth muscle cells, inhibiting apoptosis, and increasing the collagen component in the cells

[112]. However, PAEs can bind to receptors on the cell membrane in ovarian cancer, causing upregulation of cyclin D1 protein and downregulation of p21 protein, thereby promoting the proliferation of ovarian cancer cells [113]. BPA concentration in serum of patients with hysteromyoma was significantly higher than that in the control group and was positively correlated with the risk of disease. BPA can promote the occurrence of uterine fibroids by promoting the proliferation of uterine fibroid mesenchymal stem cells and accelerating the formation of cell colonies [98, 99].

10.3.6 Precocious Puberty, Menstrual Disorder, Sexual Dysfunction

Appropriate levels of estrogen are crucial to maintain female reproductive health, and EDCs can interfere with estrogen levels in vivo by mimicking or antagonizing the effects of estrogen. Since the beginning of the last century, the age of menarche in women has advanced from 16 or 17 years to less than 13 years and is also accompanied by early breast development, which may be associated with a high incidence of many other diseases such as insulin resistance, metabolic syndrome, breast cancer, and reproductive system cancer [114, 115]. Population epidemiological survey data show that mercury can cause menstrual cycle disorders in women, change the estrous cycle, and affect ovarian function [116, 117]. Excessive exposure to manganese chloride can produce severe neurotoxicity, immunotoxicity, and developmental toxicity [118–120]. Researches have shown that exposure to Mn^{2+} and its rapid entry into the third ventricle of prepubertal female rats induces a gonadotropin-releasing hormone dose-dependent stimulation of LH release. Mn^{2+} can stimulate specific puberty-related hormones, which may promote the normal beginning of adolescence, while early Mn^{2+} exposure leads to precocious puberty. Animal experiments have shown that hypothalamic dopamine (DA) is progressively and significantly decreased. However, after Mn^{2+} exposure, the mRNA levels of prolactin and pituitary transcription factor-1 increased, suggesting that Pit-1 may be a regulator of DA and prolactin [121].

PAEs (phthalic acid esters) exhibit weak estrogenic and antiestrogenic activity. In girls with premature breast development, blood PAEs concentrations were higher than that in normal girls. Phthalates are strongly associated with precocious puberty, which may be related to the antiandrogenic effect of phthalates that increases the ratio of male to female hormones [122]. Hypothyroidism occurs before puberty and may show arrested follicular development, atrophy of sexual organs, delayed menarche, etc.; if it occurs after puberty, it is characterized by oligomenorrhea or even amenorrhea, and reproductive function is inhibited. Patients have an increased incidence of combined infertility, spontaneous abortion, and teratogenesis. Thyroxine secretion and release are increased in mild hyperthyroidism, accompanied by endometrial hyperplasia, with clinical manifestations of menorrhagia,

overfrequency, and even dysfunctional uterine bleeding when hyperthyroidism is further aggravated, the secretion, release, and metabolism of thyroxine are inhibited, and the clinical manifestations are oligomenorrhea and even amenorrhea.

10.4 Prevention of EDCs, Maternal, and Infant Health Care

In order to minimize reduce the hazards of EDCs to human health, especially to female reproductive health, we should strengthen public education about EDCs knowledge, so that people can understand which substances in daily life belong to EDCs and their hazards to the human body. The public should be educated to strengthen self-protection awareness, such as not smoking, not using plastic containers to heat food, women during pregnancy to avoid contact with daily necessities or drugs related to EDCs, eat less canned and other food with preservatives, minimize the intake of fatty foods including cheese, fat meat, etc., avoid eating fish, shrimps, crabs, etc. from a potentially polluted water source, choose indoor decoration materials made with minimal or no chemical materials, reduce the number of household pesticides, detergents, repeatedly wash vegetables and fruits before eating, and avoid the use of cosmetics containing EDCs.

In short, we should avoid all EDCs containing food, drink, and daily necessities as little as possible at the present stage. Then, environmental governance should be strengthened to reduce the hazards of EDCs to human health, especially female reproductive health, from the source. In the meantime, for patients occupational exposure, the drugs for optimizing treatment are studied according to the toxicological mechanism of EDC exposure. The receptors acting on EDC, such as GPR30, a 7-transmembrane receptor, can be used in the treatment of EDC because of its high binding affinity with BPA, genistein, zeal zone, and nonylphenol [123].

There is a trend of delaying marriage and childbearing in today's society, as well as the current increase in the life span of women after birth, which requires strengthening our understanding of the mechanism of ovarian "longevity" and mastering the establishment and dynamic change process of ovarian reserve. Practical assessment of what factors fit the definition of an adverse effect includes identifying a window of sensitivity to endocrine disruption, reaching consensus on the definition of a low dose, and on the manner and timing of including a "low dose" in the design of toxicology studies.

References

1. Kavlock RJ, Daston GP, Derosa C, Fenner-Crisp P, Gray LE, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. Epa-sponsored workshop. Environ Health Perspect. 1996;104:715–40.

2. Welshons WV, Nagel SC, Vom S. Large effects from small exposures. II. Endocrine mechanisms mediating effects of bisphenol a at levels of human exposure. Endocrinology. 2006;147:s56–69.
3. Matsushima A, Kakuta Y, Teramoto T, Koshiba T, Liu XH, Okada H, Tokunaga T, Kawabata SI, Kimura M, Shimohigashi Y. Structural evidence for endocrine disruptor bisphenol a binding to human nuclear receptor ERR gamma. J Biochem. 2007;142:517.
4. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. Edc-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr Rev. 2015;36:E1–e150.
5. Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, Saal FS, Vom J. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect. 2003;111:994–1006.
6. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, von Saal FS, Welshons WV, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev. 2012;33:378–455.
7. Gan EH, Quinton. Chapter 7 – physiological significance of the rhythmic secretion of hypothalamic and pituitary hormones. Prog Brain Res. 2010;181:111–26.
8. Frederick N, Luis Miguel GS, Horvath TL, Attila Z, Necdet D, Ahmed F, Csaba L, Susanne VK, Carole L, Aimee C, Arpad P. Estrogen-induced hypothalamic synaptic plasticity and pituitary sensitization in the control of the estrogen-induced gonadotrophin surge. Reprod Sci. 2007;14:101–16.
9. Son GH, Chung S, Kim KJ. The adrenal peripheral clock: glucocorticoid and the circadian timing system. Front Neuroendocrinol. 2011;32:451–65.
10. Urbanski HF. Role of circadian neuroendocrine rhythms in the control of behavior and physiology. Neuroendocrinology. 2011;93:211–22.
11. Tian YA, Ke S, Thresia T, Robert JM, Michael AG. Transcriptional suppression of estrogen receptor gene expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (tcdd). J Steroid Biochem Mol Biol. 1998;67(1):17–24.
12. Gi HS, Sooyoung C, Han KC, Hee-Dae K, Sun-Mee B, Hankyu L, Han-Woong L, Sukwoo C, Woong S, Hyun K, Sehyung C, Kun HL, Kyungjin K. Adrenal peripheral clock controls the autonomous circadian rhythm of glucocorticoid by causing rhythmic steroid production. Proc Natl Acad Sci U S A. 2008;105(52):20970–5.
13. Essam E, Catherine AV, Dennis RS, James WO. Mechanism of toxic action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (tcdd) in cultured human luteinized granulosa cells. Reprod Toxicol. 1996;10:497.
14. Alp C, David FA. M-phase specific centrosome–microtubule alterations induced by the fungicide mbc in human granulosa cells. Mutat Res. 1997;373:139–51.
15. Zinedine A, Soriano J, Manes JJ, Toxicology C. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food Chem Toxicol. 2007;45:1–18.
16. Eskenazi B, Rauch SA, Tenerelli R, Huen K, Holland NT, Lustig RH, Kogut K, Bradman A, Sjödin A, Harley KG. In utero and childhood DDT, DDE, PBDE and PCBS exposure and sex hormones in adolescent boys: the chamacos study. Int J Hyg Environ Health. 2017;220:364–72.
17. Ornostay A, Cowie AM, Hindle M, Baker CJO, Martyniuk C. Classifying chemical mode of action using gene networks and machine learning: a case study with the herbicide linuron. Comparat Biochem Physiol Pt D. 2013;8:263–74.
18. Zhang ZB, Hu JY. Effects of p,p′-DDE exposure on gonadal development and gene expression in japanese medaka (oryzias latipes). J Environ Sci. 2008;20(3):347–52.
19. Florence E, Francoise M, Mariechantal C, Corinne L, Yvonne F, Raymond B, Daniel V, Jacques A. Chronic dietary exposure to a low-dose mixture of genistein and vinclozolin modifies the reproductive axis, testis transcriptome, and fertility. Environ Health Perspect. 2009;117(8):1272–9.

20. Constantine D, Carolyn AB. Androgens and the breast. Breast Cancer Res. 2009;11(5):212.
21. Liao DJ, Dickson RB. Roles of androgens in the development, growth, and carcinogenesis of the mammary gland. J Steroid Biochem Mol Biol. 2002;80:175–89.
22. Yeh S, Hu YC, Wang PH, Xie C, Xu Q, Tsai MY, Dong Z, Wang RS, Lee TH, Chang C. Abnormal mammary gland development and growth retardation in female mice and mcf7 breast cancer cells lacking androgen receptor. J Exp Med. 2003;198:1899–908.
23. Simanainen U, Yan RG, Walters KA, Watson G, Desai R, Jimenez M, Handelsman D. Androgen resistance in female mice increases susceptibility to dmba-induced mammary tumors. Hormones Cancer. 2012;3:113–24.
24. Andersen HR, Vinggaard AM, Rasmussen TH, Gjermandsen IM, Bonefeld-Jørgensen E. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. Toxicol Appl Pharmacol. 2002;179:1–12.
25. Gray LE Jr, Ostby J, Monosson E, Kelce WR. Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat. Toxicol Ind Health. 1999;15:48–64.
26. Monosson E, Kelce WR, Lambright C, Ostby J, Gray LE. Peripubertal exposure to the antiandrogenic fungicide, vinclozolin, delays puberty, inhibits the development of androgen-dependent tissues, and alters androgen receptor function in the male rat. Toxicol Ind Health. 1999;15:65–79.
27. Saad H, Sheikh E, Meduri G, Phrakonkham P, Bergès R, Vacher S, Djallali M, Auger J, Canivenc-Lavier MC, Perrot-Applanat MJ. Abnormal peripubertal development of the rat mammary gland following exposure in utero and during lactation to a mixture of genistein and the food contaminant vinclozolin. Reprod Toxicol. 2011;32:15–25.
28. Jugan ML, Levi Y, Blondeau J. Endocrine disruptors and thyroid hormone physiology. Biochem Pharmacol. 2010;79:939–47.
29. Tang JM, Li W, Xie YC, Guo HW, Cheng P, Chen HH, Zheng XQ, Jiang L, Cui D, Liu Y, et al. Morphological and functional deterioration of the rat thyroid following chronic exposure to low-dose pcb118. Exp Toxicol Pathol. 2013;65:989–94.
30. Schnitzler JG, Klaren PH, Bouquegneau JM, Das K. Environmental factors affecting thyroid function of wild sea bass (dicentrarchuslabrax) from European coasts. Chemosphere. 2012;87:1009–17.
31. Liu C, Ha M, Cui Y, Wang C, Yan M, Fu W, Quan C, Zhou J, Yang K. Jnk pathway decreases thyroid hormones via trh receptor: a novel mechanism for disturbance of thyroid hormone homeostasis by pcb153. Toxicology. 2012;302:68–76.
32. Kampf-Lassin A, Prendergast BJ. Acute downregulation of type II and type III iodothyronine deiodinases by photoperiod in peripubertal male and female siberian hamsters. Gen Comp Endocrinol. 2013;193:72–8.
33. Zoeller TR. Environmental chemicals targeting thyroid. Hormones. 2010;9:28–40.
34. Rogan WJ, Paulson JA, Baum C, Brock-Utne AC, Brumberg HL, Campbell CC, Lanphear BP, Lowry JA, Osterhoudt KC, Sandel MT, et al. Iodine deficiency, pollutant chemicals, and the thyroid: New information on an old problem. Pediatrics. 2014;133:1163–6.
35. Taylor PN, Okosieme OE, Murphy R, Hales C, Chiusano E, Maina A, Joomun M, Bestwick JP, Smyth P, Paradice R, et al. Maternal perchlorate levels in women with borderline thyroid function during pregnancy and the cognitive development of their offspring: data from the controlled antenatal thyroid study. J Clin Endocrinol Metab. 2014;99:4291–8.
36. Wilhelm M, Wittsiepe J, Lemm F, Ranft U, Krämer U, Fürst P, Röseler SC, Greshake M, Imöhl M, Eberwein G, et al. The duisburg birth cohort study: Influence of the prenatal exposure to PCDD/FS and dioxin-like PCBs on thyroid hormone status in newborns and neurodevelopment of infants until the age of 24 months. Mutat Res. 2008;659:83–92.
37. Xiong J, Tian L, Qiu Y, Sun D, Zhang H, Wu M, Wang J. Evaluation on the thyroid disrupting mechanism of malathion in fischer rat thyroid follicular cell line FRTL-5. Drug Chem Toxicol. 2018;41:501–8.

38. Liu C, Wang C, Yan M, Quan C, Zhou J, Yang K. Pcb153 disrupts thyroid hormone homeostasis by affecting its biosynthesis, biotransformation, feedback regulation, and metabolism. Horm Metab Res. 2012;44:662–9.
39. Sergio J, Benjamin P. Deiodinases and thyroid metabolism disruption in teleost fish. Environ Res. 2014;135:361–75.
40. Martin L, Klaassen CD. Differential effects of polychlorinated biphenyl congeners on serum thyroid hormone levels in rats. Toxicol Sci. 2010;117:36–44.
41. Berbel P, Mestre JL, Santamaría A, Palazón I, Franco A, Graells M, González-Torga A, de Escobar GM. Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. Thyroid. 2009;19:511–9.
42. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med. 1999;341:549–55.
43. Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ. Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. Clin Endocrinol. 2003;59:282–8.
44. Kennedy RL, Malabu UH, Jarrod G, Nigam P, Kannan K, Rane A. Thyroid function and pregnancy: before, during and beyond. J Obstet Gynaecol. 2010;30:774–83.
45. Krassas GE. Thyroid disease and female reproduction. J Fert Steril. 2000;74:1063–70.
46. Aghajanova L, Lindeberg M, Carlsson IB, Stavreus-Evers A, Zhang P, Scott JE, Hovatta O, Skjöldebrand-Sparre L. Receptors for thyroid-stimulating hormone and thyroid hormones in human ovarian tissue. Reprod Biomed Online. 2009;18:337–47.
47. Ji L, Ji S, Wang C, Kepp KP. Molecular mechanism of alternative p450-catalyzed metabolism of environmental phenolic endocrine-disrupting chemicals. Environ Sci Technol. 2018;52:4422–31.
48. Jiang HM, Fang ZZ, Cao YF, Hu CM, Sun XY, Hong M, Yang L, Ge GB, Liu Y, Zhang YY, et al. New insights for the risk of bisphenol A: inhibition of UDP-glucuronosyltransferases (UGTS). Chemosphere. 2013;93:1189–93.
49. Nakanishi T, Kohroki J, Suzuki S, Ishizaki J, Hiromori Y, Takasuga S, Itoh N, Watanabe Y, Utoguchi N, Tanaka K. Trialkyltin compounds enhance human CG secretion and aromatase activity in human placental choriocarcinoma cells. J Clin Endocrinol Metab. 2002;87:2830–7.
50. Kelleher AM, Milano-Foster J, Behura SK, Spencer T. Uterine glands coordinate on-time embryo implantation and impact endometrial decidualization for pregnancy success. Nat Commun. 2018;9:2435.
51. Sandeep P, Hantak AM, Bagchi IC, Bagchi MK. Minireview: steroid-regulated paracrine mechanisms controlling implantation. J Mol Endocrinol. 2014;8:1408–22.
52. Chandra Y, Madhu C, Sathishkumar K. Calcitonin gene-related family peptides in vascular adaptations, uteroplacental circulation, and fetal growth. J Curr Vasc Pharmacol. 2013;11 (5):641–54.
53. Murphy M, Reid K, Ford M, Furness JB, Bartlett PF. Fgf2 regulates proliferation of neural crest cells, with subsequent neuronal differentiation regulated by lif or related factors. Development. 1994;120:3519–28.
54. Tsatsaris V, Tarrade A, Merviel P, Garel JM, Segond N, Jullienne A, Evain-Brion D. Calcitonin gene-related peptide (CGRP) and CGRP receptor expression at the human implantation site. J Clin Endocrinol Metab. 2002;87:4383–90.
55. Wang YF, Lafont AG, Lee YC, Hwang P. A novel function of calcitonin gene-related peptide in body fluid cl-homeostasis. Proc R Soc B Biol Sci. 2016;283:20160684.
56. Yallampalli C, Chauhan M, Endsley J, Sathishkumar K. Calcitonin gene related family peptides: importance in normal placental and fetal development. Adv Fetal Neonat Physiol. 2014;814:229–40.

57. Chauhan M, Betancourt A, Balakrishnan M, Yallampalli U, Dong Y, Fox K, Belfort M, Yallampalli C. Impaired vasodilatory responses of omental arteries to cgrp family peptides in pregnancies complicated by fetal growth restriction. J Clin Endocrinol Metab. 2016;101 (8):2984–93.
58. Herbst AL, Scully R. Adenocarcinoma of the vagina in adolescence. A report of 7 cases including 6 clear-cell carcinomas (so-called mesonephromas). Cancer. 1970;25:745–57.
59. Ruthann MG, Kumiko I, Elizabeth EH. Diethylstilbestrol revisited: a review of the long-term health effects. Ann Intern Med. 1995;122:778–88.
60. Newbold RR, Jefferson WN, Padilla-Banks E. Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. Reprod Toxicol. 2007;24:253–8.
61. Soto A, Vandenberg L, Sonnenschein CJ, Pharmacology C. Does breast cancer start in the womb? Toxicology. 2010;102:125–33.
62. Witorsch RJ. Low-dose in utero effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. Food Chem Toxicol. 2002;40:905–12.
63. Ehrlich S, Williams PL, Missmer SA, Flaws JA, Ye X, Calafat AM, Petrozza JC, Wright D, Hauser R. Urinary bisphenol a concentrations and early reproductive health outcomes among women undergoing ivf. Hum Reprod. 2012;27:3583–92.
64. Mok-Lin E, Ehrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, Ye X, Hauser R. Urinary bisphenol a concentrations and ovarian response among women undergoing ivf. Int J Androl. 2010;33:385–93.
65. Bloom MS, Kim D, Vom Saal FS, Taylor JA, Cheng G, Lamb JD, Fujimoto VY. Bisphenol a exposure reduces the estradiol response to gonadotropin stimulation during in vitro fertilization. Fertil Steril. 2011;96:672–7.
66. Ehrlich S, Williams PL, Hauser R, Missmer SA, Peretz J, Calafat AM, Flaws JA. Urinary bisphenol a concentrations and cytochrome p450 19 a1 (cyp19) gene expression in ovarian granulosa cells: an in vivo human study. Reprod Toxicol. 2013;42:18–23.
67. Lee SH, Kang SM, Choi MH, Lee J, Park MJ, Kim SH, Lee WY, Hong J, Chung BC. Changes in steroid metabolism among girls with precocious puberty may not be associated with urinary levels of bisphenol A. Reprod Toxicol. 2014;44:1–6.
68. Eleni K, Antonis C, Sarantis L, Eleni P, Frangiscos E, Michael K, Sotiria P, Dimitrios P, Evanthia DK. Endocrine disruptors and polycystic ovary syndrome (pcos): Elevated serum levels of bisphenol a in women with pcos. J Clin Endocrinol Metab. 2011;96:E480.
69. Anette KK, Inger N, Janneche US, Wenche F, Ann-Lill H. In vitro reproductive toxicity of polychlorinated biphenyl congeners 153 and 126. Reprod Toxicol. 1998;12(6):575–80.
70. Ema M, Kurosaka R, Amano H, Ogawa Y. Comparative developmental toxicity of butyltin trichloride, dibutyltin dichloride and tributyltin chloride in rats. J Appl Toxicol. 2010;15:297–302.
71. Smarr MM, Kannan K, Buck Louis GM. Endocrine disrupting chemicals and endometriosis. Fertil Steril. 2016;106:959–66.
72. Pauwels A, Schepens PJ, D'Hooghe T, Delbeke L, Dhont M, Brouwer A, Weyler J. The risk of endometriosis and exposure to dioxins and polychlorinated biphenyls: a case-control study of infertile women. Hum Reprod. 2001;16:2050–5.
73. Brenda E, Paolo M, Marcella W, Steven S, Paolo V, David O, Needham LL, Patterson DG, Paolo B, Nicoletta G. Serum dioxin concentrations and endometriosis: a cohort study in seveso, Italy. Environ Health Perspect. 2002;110:629–34.
74. Rier SE, Turner WE, Dan CM, Morris R, Lucier GW, Clark G. Serum levels of TCDD and dioxin-like chemicals in rhesus monkeys chronically exposed to dioxin: correlation of increased serum pcb levels with endometriosis. Toxicol Sci. 2001;59:147–59.
75. Eskenazi B, Mocarelli P, Warner M, Samuels S, Vercellini P, Olive D, Needham LL, Patterson DG Jr, Brambilla P, Gavoni N, et al. Serum dioxin concentrations and endometriosis: A cohort study in seveso, italy. Environ Health Perspect. 2002;110:629–34.

76. Kim SH, Cho S, Ihm HJ, Oh YS, Heo SH, Chun S, Im H, Chae HD, Kim CH, Kang BM. Possible role of phthalate in the pathogenesis of endometriosis: in vitro, animal, and human data. J Clin Endocrinol Metab. 2015;100(12):2478.
77. Missmer SA, Hankinson SE, Donna S, Barbieri RL, Michels KB, Hunter DJ. In utero exposures and the incidence of endometriosis. Fertil Steril. 2004;82:1501–8.
78. Upson K, Sathyanarayana S, Scholes D, Holt VL. Early-life factors and endometriosis risk. Fertil Steril. 2015;104:964–71.
79. Palioura E, Diamanti-Kandarakis E. Polycystic ovary syndrome (pcos) and endocrine disrupting chemicals (edcs). Rev Endocr Metab Disord. 2015;16:365–71.
80. Zhou W, Liu J, Liao L, Han S, Liu JJ. Effect of bisphenol a on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. Mol Cell Endocrinol. 2008;283:12–8.
81. Déchaud H, Ravard C, Claustrat F, Perrière ABDL, Pugeat MJ. Xenoestrogen interaction with human sex hormone-binding globulin (hshbg) 1. Steroids. 1999;64:328–34.
82. Hanioka N, Jinno H, Nishimura T, Ando M. Suppression of male-specific cytochrome p450 isoforms by bisphenol a in rat liver. J Arch Toxicol. 1998;72:387–94.
83. Yokota H, Iwano H, Endo M, Kobayashi T, Inoue H, Ikushiro S, Yuasa AJ. Glucuronidation of the environmental oestrogen bisphenol a by an isoform of udp-glucuronosyltransferase, ugt2b1, in the rat liver. Biochem J. 1999;340(Pt 2):405.
84. Alice M, Simon D, Frédéric L, Arnaud P, Alain P, Nathalie P, Justine B-M, Thierry P, Hervé G, Pascal GP, Laïla M-L. Low doses of bisphenol a induce gene expression related to lipid synthesis and trigger triglyceride accumulation in adult mouse liver. Hepatology. 2011;55(2):24685.
85. Barrett ES, Sobolewski M. Polycystic ovary syndrome: do endocrine-disrupting chemicals play a role? Semin Reprod Med. 2014;32:166–76.
86. Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. J Obstet Gynecol. 1986;67:604–6.
87. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. Hum Reprod Update. 2015;21:787–808.
88. Vabre P, Gatimel N, Moreau J, Gayrard V, Picard-Hagen N, Parinaud J, Leandri RD. Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. Environ Health. 2017;16:37.
89. Iorio R, Castellucci A, Ventriglia G, Teoli F, Cellini V, Macchiarelli G, Cecconi S. Ovarian toxicity: from environmental exposure to chemotherapy. Curr Pharm Des. 2014;20:5388–97.
90. Dragojević-Dikić S, Vasiljević M, Nikolić B, Pazin V, Tasić L, Jurisić A, Dikić S, Perisić Z. Premature ovarian failure: immunological aspects and therapeutic strategies. Vojnosanit Pregl. 2013;70:1051–5.
91. Hewlett M, Mahalingaiah S. Update on primary ovarian insufficiency. Curr Opin Endocrinol Diabetes Obes. 2015;22:483–9.
92. Tucker EJ, Grover SR, Bachelot A, Touraine P, Sinclair AH. Premature ovarian insufficiency: new perspectives on genetic cause and phenotypic spectrum. Endocr Rev. 2016;37:609–35.
93. Craig ZR, Wang W, Flaws JA. Endocrine-disrupting chemicals in ovarian function: effects on steroidogenesis, metabolism and nuclear receptor signaling. Reproduction. 2011;142:633.
94. Matikainen T, Perez GI, Jurisicova A, Pru JK, Schlezinger JJ, Ryu HY, Laine J, Sakai T, Korsmeyer SJ, Casper RF. Aromatic hydrocarbon receptor-driven bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. Nat Genet. 2001;28:355.
95. Yasushi T, Jacqueline C, Perez GI, Pru JK, Schlezinger JJ, Sherr DH, Kolesnick RN, Junying Y, Flavell RA, Korsmeyer SJ. Bax, caspase-2, and caspase-3 are required for ovarian follicle loss caused by 4-vinylcyclohexene diepoxide exposure of female mice in vivo. J Endocrinol. 2003;144:69.
96. Hunt PA, Crystal L, Mary G, Brenda M, Helen S, Alyssa M, Terry H, Vandevoort CA. Bisphenol a alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey. Proc Natl Acad Sci U S A. 2012;109:17525–30.

97. Lee SG, Kim JY, Chung J-Y, Kim Y-J, Park J-E, Oh S, Yoon Y-D, Yoo KS, Yoo YH, Kim J-M. Bisphenol a exposure during adulthood causes augmentation of follicular atresia and luteal regression by decreasing 17β-estradiol synthesis via downregulation of aromatase in rat ovary. Environ Health Perspect. 2013;121:663–9.
98. Jiping X, Yutaka O, Tetsu Y, Yutaka M, Xiaohui T, Toshihiro F, Yasushi T, Hirotaka M, Kaori K, Yuji T, Osamu T. Bisphenol a induces apoptosis and g2-to-m arrest of ovarian granulosa cells. Biochem Biophys Res Commun. 2002;292:456–62.
99. Jackye P, Craig ZR, Flaws JA. Bisphenol a inhibits follicle growth and induces atresia in cultured mouse antral follicles independently of the genomic estrogenic pathway. Biol Reprod. 2012;87:63.
100. Ge W, Li L, Dyce PW, De Felici M, Shen W. Establishment and depletion of the ovarian reserve: physiology and impact of environmental chemicals. Cell Mol Life Sci. 2019;76 (9):1729–46.
101. La-Sala G, Farini DF, Massimo DF. Proapoptotic effects of lindane on mouse primordial germ cells. J Toxicol Sci. 2009;108:445.
102. Crystal L, Mary G, Brenda M, Ping Y, Yunfei L, Terry H, Hunt PA. Gene expression in the fetal mouse ovary is altered by exposure to low doses of bisphenol a. Biol Reprod. 2011;84:79–86.
103. Zhang XF, Zhang LJ, Feng YN, Chen B, Feng YM, Liang GJ, Li L, Shen WJ. Bisphenol a exposure modifies DNA methylation of imprint genes in mouse fetal germ cells. Mol Biol Rep. 2012;39:8621–8.
104. Li Y, Zhang W, Jin L, Wang W, Hong L, Zhu J, Weng S, Xiao S, Wu TJ. Prepubertal bisphenol a exposure interferes with ovarian follicle development and its relevant gene expression. Reprod Toxicol. 2014;44:33–40.
105. Holm JB, Mazaud-Guittot S, Danneskiold-Samsøe NB, Chalmey C, Jensen B, Nørregård MM, Hansen CH, Styrishave B, Svingen T, Vinggaard AM. Intrauterine exposure to paracetamol and aniline impairs female reproductive development by reducing follicle reserves and fertility. Toxicol Sci. 2016;150:178.
106. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
107. Fenton SE. Endocrine-disrupting compounds and mammary gland development: early exposure and later life consequences. Endocrinology. 2006;147:S18–24.
108. Jenkins S, Betancourt AM, Wang J, Lamartiniere CA. Endocrine-active chemicals in mammary cancer causation and prevention. J Steroid Biochem Mol Biol. 2012;129:191–200.
109. Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol a exposure. Reprod Toxicol. 2007;23:383–90.
110. Marcella W, Brenda E, Steven JS, Larry LN, Paolo B, Paolo M. Serum dioxin concentrations and breast cancer risk in the seveso women. Epidemiology. 2002;110:625–8.
111. Greenlee WE, Hushka LJ, Hushka DR. Molecular basis of dioxin actions: evidence supporting chemoprotection. Toxicol Pathol. 2001;29:6.
112. Jin HK, Kim SH, Oh YS, Ihm HJ, Chae HD, Kim CH, Kang BM. In vitro effects of phthalate esters in human myometrial and leiomyoma cells and increased urinary level of phthalate metabolite in women with uterine leiomyoma. Fertil Steril. 2017;107:1061–9.
113. Minah P, Kyunga H, Hyerim L, Borim Y, Euibae J, Kyungchul C. Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. Mol Med Rep. 2011;5 (3):761–6.
114. Costa EM, Spritzer PM, Hohl A, Bachega TA. Effects of endocrine disruptors in the development of the female reproductive tract. Arq Bras Endocrinol Metabol. 2014;58:153–61.

115. Buttke DE, Sircar K, Martin C. Exposures to endocrine-disrupting chemicals and age of menarche in adolescent girls in nhanes (2003-2008). Environ Health Perspect. 2012;120:1613–8.
116. Rowland AS, Baird DD, Weinberg CR, Shore DL, Shy CM, Wilcox AJ. The effect of occupational exposure to mercury vapour on the fertility of female dental assistants. Occup Environ Med. 1994;51:28–34.
117. Davis B, Price H, O'Connor R, Fernando R, Rowland A, Morgan DJ. Mercury vapor and female reproductive toxicity. Toxicol Sci. 2001;59:291–6.
118. Tsuchiya H, Shima S, Kurita H, Ito T, Kato Y, Kato Y, Tachikawa S. Effects of maternal exposure to six heavy metals on fetal development. Bull Environ Contam Toxicol. 1987;38:580–7.
119. Laudanski T, Sipowicz M, Modzelewski P, Bolinski J, Szamatowicz J, Razniewska G, Akerlund M. Influence of high lead and cadmium soil content on human reproductive outcome. Int J Gynaecol Obstet. 1991;36:309–15.
120. Pine M, Lee B, Dearth R, Hiney JK, Dees WL. Manganese acts centrally to stimulate luteinizing hormone secretion: a potential influence on female pubertal development. Toxicol Sci. 2005;85:880–5.
121. Pine M, Lee B, Dearth RHiney JK, Dees WL. Manganese acts centrally to stimulate luteinizing hormone secretion: a potential influence on female pubertal development. Toxicol Sci. 2005;85:880.
122. Colón I, Caro D, Bourdony CJ, Rosario O. Identification of phthalate esters in the serum of young puerto rican girls with premature breast development. Environ Health Perspect. 2000;108:895–900.
123. Helle Raun A, Anne Marie V, Thomas Hoj R, Irene Marianne G, Cecilie E. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. Toxicol Appl Pharmacol. 2002;179:1–12.

Chapter 11
Definition and Multiple Factors of Recurrent Spontaneous Abortion

Xiaolin La, Wenjuan Wang, Meng Zhang, and Li Liang

Abstract Recurrent spontaneous abortion (RSA) is usually defined as three or more spontaneous abortions prior to 20–28 weeks gestation. RSA affects approximately 2–5% of all women of childbearing age, and it brings tremendous psychological and psychiatric trauma to the women and also results in economic burden. The causes could be female age, anatomical and chromosomal abnormalities, genetic, endocrinological, placental anomalies, infection, smoking and alcohol consumption, psychological factor, exposure to environmental factors such as heavy metal, environment pollution, and radiation.

Keywords Recurrent spontaneous abortion · Molecular pathophysiology · Nightwork and long work hours · Toxic substance · Radiation exposure · Psychological stress · Medical or surgical abortion · Microelements and RSA

11.1 Introduction of Spontaneous Miscarriage

Pregnancy loss or miscarriage refers to the termination of pregnancy before the fetus survives and is the most common pregnancy complication in obstetrics. Abortion is categorized as sporadic abortion and recurrent spontaneous abortion according to the frequency of occurrence. The definition of RSA (recurrent spontaneous abortion) is various in different countries and regions because RSA is a frequency descriptive disease. The ASRM (American Society for Reproductive Medicine) and ESHRE

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(European Society of Human Reproduction and Embryology) define RSA as two or more clinical pregnancy losses with an identical partner, the incidence is approximately 5% [1]. The Chinese Medical Association Obstetrics and Gynecology Branch defines RSA as three or more consecutive pregnancy loss before 28 weeks gestation, and The Royal College of Obstetricians and Gynaecologists (RCOG) defines RSA as three or more pregnancy loss prior to 24 weeks [2, 3].

RSA affects 1–2% of couples. The risk of pregnancy loss is more in the early gestations. The incidence of RSA is hard to estimate because of the differences in definition and criteria in different countries and regions. There is no valid estimate of the prevalence of RSA because of lack of worldwide registration of RSA and miscarriages, furthermore, many early pregnancy losses were ignored because it was not recognized or treated in the hospital. The prevalence of RSA is found between 0.6% and 2.3% from various studies [1, 4].

11.2 Pathology

The etiology of RSA is complicated, and it can be a simple factor or a combination of multiple factors. The known causes include genetic factors, reproductive tract anatomical abnormalities, immune dysfunction, endocrine abnormalities, infectious factors, thrombotic factors, the external environment, and other factors. Despite a thorough evaluation of these causes, about 50% of patients' etiology still unexplained [5].

11.2.1 Genetic Factors

Chromosomal abnormalities are the most common causes of RSA, 2–5% RSA couples have abnormal chromosome chromosomal structural abnormalities, including chromosomal translocations, chimeras, deletions, or inversions. Chromosomal equilibrium translocation and Robertson translocations are the two most common chromosomal structural abnormalities. The balanced translocation chromosome phenotypes were clinically normal, but the study found that the risk of miscarriage after pregnancy increased significantly, and the offspring more susceptible to abnormal. Robertsonian translocations and non-homologous Robertsonian translocations are based on chromosomes in which translocation occurs. The incidence of Robertsonian translocation in the general population is about 1/1000, 1.1% in patients with RSA. Robertson translocations in homologous chromosome cannot produce gametes theoretically, but the germ cells of the non-homologous Robertson translocations can produce 6 gametes after meiosis process, of which 1/6 is normal karyotype and 1/6 is a balanced translocation carrier after fertilization [6].

Embryo chromosomal abnormalities are the most common cause of RSA, mostly caused by teratogenic factors in the environment such as radiation, viruses, or drugs acting on germ cells or early developmental embryos. About half of the embryos in

sporadic early spontaneous abortion have chromosomal abnormalities. In addition, it has been reported that the earlier an abortion occurs, the higher the incidence of embryonic chromosomal abnormalities.

11.2.2 Immune Factors

Recently, reproductive immunity studies suggested that immune dysfunction accounts for about 50% causes of RSA. Immune abortion can be divided into autoimmune RSA and alloimmune RSA.

1. Autoimmune RSA: (1) Tissue non-specific autoantibody production. (2) Tissue specific autoantibody, such as anti-sperm antibodies, anti-thyroid antibodies, etc.
2. Alloimmune RSA: (1) Innate immune disorders: including natural killer (NK) cell number and activity, macrophage dysfunction, dendritic cell dysfunction, complement system abnormalities. (2) Acquired immune disorders: including blocking antibody deficiency, T, B lymphocyte abnormalities, helper T lymphocytes, Th1/Th2 cytokine abnormalities, etc.

Antiphospholipid antibody syndrome (APS) is a non-inflammatory autoimmune disease characterized by producing a large number of antiphospholipid antibodies (APL), including ACA, LA, and anti-β 2 GP1 antibodies. Its clinical manifestations include arteriovenous thrombosis, pathological pregnancy, and reduced platelet count. APS is one of the most important and treatable causes of RSA. Five percent to twenty percent of patients with RSA can detect antiphospholipid antibodies [2], and the live birth rate would decline to 10% in untreated RSA patients. Regarding the relationship between thyroid autoantibodies and abortion, evidence-based medical evidence indicated that the correlation between thyroid autoantibodies and abortion had statistical significance. Studies have found that RSA patients had higher thyroid autoantibodies positive rate, and other studies indicate that women with thyroid autoantibodies have increased incidence of RSA [7].

11.2.3 Infectious Factors

The inflammatory response caused by any pathogen may theoretically lead to miscarriage, but such abortions are mostly sporadic abortions. Among patients with recurrent spontaneous abortion, the pathogens with higher detection rates are chlamydia, mycoplasma, toxoplasma, herpes simplex virus, rubella virus, cytomegalovirus, and human cytomegalovirus [8].

11.2.4 Anatomical Factors

Uterine anatomical abnormalities include: (1) congenital uterine malformation: mainly including the double uterus, the double-horned uterus, the saddle-shaped uterus, the single-horned uterus, the mediastinal uterus and (2) cervical incompetence refers to a painless spontaneous dilatation of the cervix and is a common cause of second trimester pregnancy failure.

Intrauterine adhesions (IUA) are uterine diseases that are common in gynecology, having adverse effects on fertility and poor therapeutic effects, which seriously affect women's reproductive physiology and mental health. According to reports in the literature, the incidence of IUA caused by repeated abortions and curettage is as high as 25–30%, which has become the main cause of decreased menstrual flow and secondary infertility [9].

11.2.5 Uterine Fibroids

Uterine fibroids may increase the incidence of spontaneous abortion and is an important cause of recurrent miscarriage. Submucosal fibroids can affect the implantation of fertilized eggs leading to early abortion, larger submucosal fibroids protrude into the uterine cavity, or the intermuscular fibroids are too large to deform the body cavity or be mechanically compressed, leading to abnormal function or influence of endometrium. The vascular structure of the local endometrium causes endometrial insufficiency to cause abortion; the fibroids may interfere with the formation of the placenta and the perfection of the uteroplacental circulation if the embryo just happens to implant the fibroid, which would lead to loss of pregnancy.

11.2.6 Endometriosis

Prostaglandin secretion in patients with endometriosis increased and stimulates uterine contractions that interfere with implantation of a fertilized egg; endometriosis can cause non-specific inflammation of the endometrium, which has cytotoxicity to the embryo; Furthermore, endometriosis is an autoimmune disease. The ectopic endometrium can stimulate the body to produce anti-endometrial antibodies, which could cause abortion through interfering with the process of fertilized egg implantation [10].

11.2.7 Environmental Factors

The environment is the material basis for human survival and development. Reproductive health is an important guarantee for human beings to prosper. In the long process of evolution, human beings adapt to the ecological environment and transform it, and the environment and human reproductive health are in a dynamic equilibrium state [11].

There is a continuous and complex relationship between people and the environment that is interdependent and interacting. The reproductive process affected by the environment could occur in any step including gamete formation, fertilization, zygote transport in the reproductive tract, implantation process, fetal maturation, childbirth, neonatal adaptation to childhood growth and development, sexual maturity. Reproductive health damage, such as reproductive dysfunction or adverse pregnancy outcomes, may occur when exposed to adverse environmental factors.

11.2.7.1 Toxic Substance and RSA

The environment consists of three main compartments: water, air, and soil, which coexist in a condition of dynamic balance. Although initially, one kind of pollutant may be distributed predominantly in one compartment, generalized it may translate, particularly for persistent pollutants or pollutants whose degradation products persist [12]. Many factors may affect reproduction, which can be incorporated into the body via ingestion, inhalation, and transcutaneously. The chemical pollutants can be divided into four categories. One of them consists of many natural chemicals, including nitrates, which are normal dietary components and mercury intake via fish consumption. Too much nitrates and mercury accumulated in food or water can lead to spontaneous abortions [13–15]. Another kind of chemical pollutants is natural fungal or plant toxins in crops, of which cycasins and aflatoxins are prime examples. Toxic materials in the diets can affect reproductive in many factors, like causing abortions, affecting sexual desire, estrus, or disturbing spermatogenesis and oogenesis, causing abnormal mating behavior, birth defects, and delaying the second pregnancy [16]. Fed aflatoxin-contaminated peanuts cause abortion in ruminants [17, 18]. In animal study, a large amount of plant toxins are potent abortifacients. In livestock, they may cause embryonic loss or fetal death through crossing the maternal–fetal interface and interfere with embryonic and fetal growth. The plants include astragalus spp, locoweed, pinus spp, pinus ponderosa, tetradymia glabrata, littleleaf horsebrush, veratrum californicum, false hellebore, nitrate-containing plants, aspidosperma pyrifolium, enterolobium contortisiliquum, *S. obovatum*, *S. fissuratum*, etc. [16, 19]. Many toxins consumed by animals may undergo biotransformation and are secreted in milk [20–22]**.** Cats that drink milk from cows eating locoweed have produced symptoms of locoweed poisoning [23]. Given that milk is widely consumed and as a source of nutrients, especially during pregnancy, when human feed on such poisoned plants or milk, reproductive

function may be affected and abortion may occur. The third kind of chemical pollutants contains many complex inorganic and organic mixtures, which comprise a large number of undefined and defined components. Variations in menstrual (single or continuous cycles, changes in luteal phase or follicular phase, missed periods, and abnormal bleeding) and ovarian function have been observed after drinking water disinfection byproducts (DBPs), chlordibromomethane, and fish polluted by polychlorinated biphenyls (PCBs), endocrine-active compounds such as TCDD, or working in industry covered of all kinds of chemicals like lead and ethylene glycol ethers [24]. The functional variations are correlated with an underlying perturbation of hormones [25]. Chlorinated hydrocarbons (CHCs) are reported to store in different organs, especially the fatty tissue; exhibit hormone like activity; and induce immunological changes [26]. Embryo toxic effects of PCP have been confirmed in numerous animal feed studies and may also contribute to repeated miscarriages in humans [26]. Benzene, toluene, xylene, and formaldehyde are airborne toxicities in air pollutants causing embryotoxicity and abortion [27]. Significant associations with spontaneous abortion were found for exposure to organic solvents such as toluene and formalin and tetrachloroethylene [28–30]. Abnormal development of fetal, fetal loss, and neonatal death were associated with heavy metals, like lead, arsenic, and mercury exposure influencing endocrine during pregnancy [31]. The last group consists of synthetic chemicals–agricultural chemicals, notably pesticides, fertilizers, food additives, heavy metals and plasticizers, and prophylactic drugs or drugs of abuse. Teratogens may result in fetotoxicity and malformations at maternally toxic levels, then produce chromosome aberrations which increase the abortion rate with abnormal karyotype. But if they act after fertilization, the aberrations will be sporadic against a normal background. Working in conditions that use many kinds of pesticides (e.g., phenoxy acetic acids, carbamates, and organophosphates) may elevate risk for spontaneous abortion [32, 33]. Preconception exposures lead to high risk of chromosomal anomalies. Postconception exposure cannot cause chromosomal anomalies, but specific pesticides will damage the fetus or fetus–placenta complex [33].

In addition, the previous available statistics of Syria, women who exposed to chemical attacks can increase incidence of spontaneous abortion, stillbirth, and birth defects [34]. Because of the developing brain is susceptible to neurotoxic invasion, the effects of chemical could be seen between children and pregnant women [35].

In conclusion, it is necessary to guide the childbearing women to consider any potentially harmful occupational exposures, avoid touching the known or suspected risk factors, taking measures to reduce the toxicant concentration in the workplace.

11.2.7.2 Radiation Exposure and RSA

Radiation is the energy that travels through some material or space and is categorized as nonionizing or ionizing. Ionizing radiation consists of either particulate or electromagnetic energy, can be described as “has enough energy to remove tightly bound

electrons from atoms, thus creating ions." Examples of particulate energy include heat or light from the sun, X-rays from an X-ray tube, and gamma rays from radioactive elements. Nonionizing radiation can be described as "has enough energy to move around atoms in a molecule or cause them to vibrate, but cannot remove electrons"; examples include visible light, infrared (IR), microwave (MW) and extremely low frequency (ELF), and so on [36].

Negative effects of radiation on male and female reproduction have been confirmed in various animal species and human beings. The reproductive system of human beings is sensitive to radiation, different duration and dose rate can cause temporary or permanent sterility [37]. The risk of fetal damage at doses lower than 1 mGy is similar to a fetus that is exposed to no radiation. Sensitivity to radiation during pregnancy depends on different developmental stages. Generally, the key stages in pregnancy are pre-implantation or blastogenesis (0–2 weeks), organogenesis (3–8 weeks), and fetal development from the ninth week until birth [38–40]. If the embryo is exposed to radiation before implantation, it will exist or miss [39, 41, 42]. During the first 2 weeks after conception, a small dose like 100–200 mGy may destroy an embryo [43]. After that stage, the critical value for fetal death increases to 250–500 mGy, with the fetal death threshold increasing throughout gestation as the fetal develops [43]. The estimated radiation dose necessary to kill all embryos or fetal <18 weeks' gestation is 5000 mGy [44]. At term, the fetal risk equivalents to the pregnant woman's risk, as 20,000 mGy [43, 45]. Differences in radiation doses that cause harm to the fetus may because of the protect from a pregnant woman's surrounding such as soft tissues, amniotic fluid and uterus, which may prevent alpha and beta particles from penetration; however, there are also some radiations, like gamma and X-rays directed toward the abdomen of a pregnant woman can reach and harm the fetus. In addition, whatever radioactive ions get into a pregnant woman's body in any way, the placenta can transfer it, and radioactive material that accumulates in the bladder may result in radiation exposure to the fetus.

Ionizing radiation exposure consists of occupation-related exposure and non-occupational exposure. The primary biological effect of shortwaves, microwaves, ultrasound, and various superficial electric currents which are used in physiotherapy is thought to be via hyperthermia. According to the report, mothers who were exposed to radiation in their workplace, especially monitored within 6 months of conception, will increase risk of early miscarriage. But there was no significant correlation with stillbirths and second trimester miscarriage [46, 47]. However, long time exposure from microwave- and radio-frequency electromagnetic radiation may cause late spontaneous abortions [48]. There are different evidence about different influence of physiotherapists' occupational exposure to radiofrequency electromagnetic fields from shortwave and microwave diathermy devices may relate to the duration of exposure, the dose as well as differences in different devices and different manufacturers are the most important parameters for determining the impact on the fetus [10]. We also should pay more attention to the distance from therapeutic diathermy device and the intensity and maintain time of RF EMFs exposure [49]. For pregnant radiation workers, the upper limits of the maternal dose should be less than 5 mGy. Similarly, during the gestational period,

the fetal dose for radiation workers should be less than 1 mGy. This dose is the same for members of the public who are incidentally exposed to radiation. What's more, therapeutic abortion should be considered if a dose of 100 mGy is often mentioned to a developing fetus. However, this point is still no final conclusion [50, 51], somebody suggest that a lower fetal dose (>200 mGy) could be considered as therapeutic abortion.

For non-occupational exposure, pregnant women exposed to irradiant diagnostic procedures cannot increase the risk of malformation. The average doses from radiography and CT for fetal from 0.05 ± 0.01 mGy to 22.94 ± 1.28 mGy have no obvious effect on pregnancy outcome. Fetal radiation exposures reach the level (50 mGy) may increase risk for congenital anomalies [38, 52]. There is no evidence to suggest that women with Graves' disease who received iodine-131 treatment before pregnancy will do harm to the fetal. And no significant risk of abnormal fetal development has been observed on pregnant women with Graves' disease after a 6-month period of iodine-131 treatment [53].

There is no significant difference between cancer patients who received radiotherapy and subsequently became pregnant and healthy women [54, 55]. Childhood radiation will not increase the risk of abortion or congenital malformation of the offspring, but radiotherapy treatment can lead to ovarian failure, which results in infertility, incidence of stillbirth, and low birth weight among offspring [56]. For most people, air travel cosmic radiation is negligible. However, aircrew or frequent flyers associated with cosmic radiation exposure including galactic cosmic radiation and solar particle events may exceed these limits and have an increased incidence of miscarriage [57, 58]. The Federal Aviation Administration and the International Commission on Radiological Protection consider aircrew to be occupationally exposed to ionizing radiation [20–22].

As to nonionizing radiation, mid- and high-intensity electromagnetic radiation may reduce the fertilization rate in mice, thus reducing the possibility of embryo implantation [59]. However, there remains little evidence of association between exposure to electrical and adverse pregnancy outcomes [37]. Decreased UV exposure is associated with low vitamin D and spontaneous abortion [60, 61], as vitamin D plays a crucial part in maternal–fetal immune tolerance [62]. Laser-assisted hatching (LAH), the most promising embryo hatching technology, is connected with a higher clinical pregnancy rate, embryo implantation rate, and multiple pregnancy rate in women with cryopreserved-thawed embryos. But it cannot increase live birth rates and miscarriage rates [63]. Low-level radiofrequency (3 kHz to 300 GHz) energy exposures from cellular telephone, radar, and radio/television-transmission have no adverse health effects [64]. Ultrasound examination is safety, but long time ultrasound exposure may induce human chorionic villus cell apoptosis during early pregnancy by increasing the Bax/Bcl-2 protein ratio then increase the abortion rate [65]. In mice, ultrasound could increase tumor necrosis factor-alpha release from the decidua ,decrease TGF-beta 2-mediated suppressive activity, increase the expression of adhesion molecules facilitating the raise of inflammatory cells to the fetomaternal interface, then increase the abortion rate [66, 67].

In conclusion, according to the Committee Opinion No. 723: Guidelines for Diagnostic Imaging During Pregnancy and Lactation [40]. (1) Ultrasonography and magnetic resonance imaging (MRI) are not related to risk and may be the best choice for the pregnant woman, but they should be used when they are expected to answer a relevant clinical question or provide medical benefit to the patient. (2) With few exceptions, radiation exposure through radiography, computed tomography scan is at a dose much lower than the exposure associated with fetal harm. If these techniques are necessary in addition to ultrasonography or MRI or are more necessary for the diagnosis, they can also be considered. (3) The use of gadolinium contrast with MRI should be limited.

Prevention measures for pregnant women should on account of the following three principles: keeping a safe distance, protecting themselves from exposure, and avoiding ingestion of food and water polluted by radioactive particles from its surroundings.

11.2.8 Age and Psychological Factors

High maternal age is one of the strongest factors of RSA. The risk of pregnancy loss is lower in women aged 20–35 years old and increased rapidly after the age of 40 years [14, 68].

11.2.9 Prethrombotic State

The prethrombotic state (PTS) is a blood coagulation state caused by a decrease in the concentration of blood coagulation inhibitors, which has not yet reached the level of thrombus formation or a small amount of thrombus that has formed in a dissolved state.

Clinical prethrombotic states include both congenital and acquired types. (1) The congenital prethrombotic state is caused by genetic mutations related to coagulation and fibrinolysis, such as mutations in factor V and factor II (prothrombin) and deficiency of protein S. Meta-analysis showed that late spontaneous abortion was closely related to congenital thrombosis caused by mutations in factor V and factor II (prothrombin) and protein S deficiency [69–72]. However, mutations in factor V and factor II (prothrombin) are rare in the Han population. (2) The acquired prethrombotic state mainly includes antiphospholipid syndrome (APS), acquired hyperhomocysteinemia, and various other diseases that cause hypercoagulability of blood.

At present, the specific mechanism of spontaneous abortion caused by the prethrombotic state has not been completely clarified. It is generally believed that the hypercoagulable state during pregnancy changes the blood flow state of the uterus placenta, and it is easy to form local microthrombus or even cause a placental

infarction, which causes the blood supply of placental tissue to decrease. The embryo or fetal ischemia and hypoxia eventually lead to miscarriage of the embryo or fetus. Unfortunately, women with prethrombotic conditions have no obvious clinical manifestations, and there are no clear diagnostic criteria for their hematologic examination.

11.2.10 Endocrinology of RSA

11.2.10.1 PCOS

PCOS is a complicated disease including interactions between the pancreas, hypothalamus/pituitary, ovary, liver, and adipose tissue. The causes of RSA in patients with PCOS may have many mutually related factors [73], including obesity, hyperinsulinemia, hyperandrogenism, insulin resistance (IR), poor endometrial receptivity, and elevated LH levels. An estimation indicated that 40% of pregnancies with PCOS will lead to spontaneous abortion [74].

Obesity affects female reproductive function by way of hyperinsulinemia, which in turn affects androgen production. Many researchers believe that IR is a key factor in explaining the association among obesity, PCOS, and RSA [75, 76]. In addition, many studies have shown that IR may be associated with hyperhomocysteinemia [77, 78]. Recent studies have highlighted that low fibrinolysis and high levels of plasminogen activator inhibitor-1 (PAI-1) may be related to repeated pregnancy loss in patients with PCOS [79, 80].

11.2.10.2 Diabetes

Pre-pregnancy diabetes includes type 1, type 2, and other types , accounting for 0.5–1% of all pregnant women [81]. Many investigations have shown that patients with pre-pregnancy diabetes have a clinically significant increased risk of spontaneous abortion, premature birth, hypertension, and surgical delivery. However, the risk of pregnancy loss may increase by other known maternal risk factors, such as advanced maternal age, previous history of miscarriage, alcohol and smoke consumption, overweight.

A retrospective observational study showed that patients with abortion had higher HbA1c levels than those with a good pregnancy outcome. In addition, the study also indicated that maternal age and HbA1c were important predictors of abortion. At the same time, the study also found that pregnancy loss during early pregnancy was associated with overweight/obesity, hypertension, unplanned pregnancy, long-term diabetes, and diabetic vascular complications. This was not statistically significant, but this trend can be seen from the data [82].

11.2.10.3 Hyperthyroidism

Occurrence of hyperthyroidism is approximately 0.1–0.4% of pregnancies [83]. Pregnancies with untreated hyperthyroidism have higher risk of miscarriage, congestive heart failure, thyroid storm, premature delivery, pre-eclampsia, fetal growth restriction, perinatal morbidity, and mortality [84, 85]. Treatment of Graves' hyperthyroidism during pregnancy to achieve adequate metabolic control can improve pregnancy outcome [86]. However, it should be noticed that hyperthyroidism is not an independent factor in RSA.

11.2.10.4 Hypothyroidism

The cause of hypothyroidism in pregnancies is chronic autoimmune thyroiditis (Hashimoto's thyroiditis), which affects about 0.5% of pregnant women [87]. Other causes of hypothyroidism were endemic iodine deficiency (ID), previous radioactive iodine therapy, and thyroidectomy.

11.2.10.5 Luteal Phase Deficiency

Luteal phase deficiency (LPD) may reduce the production of progesterone in the luteal phase due to poor follicular formation and a decrease in the response of the endometrium to progesterone. LPD has other causes including stress, exercise, weight loss, hyperprolactinemia [88]. Thirty-five percent of women with recurrent abortion have luteal dysfunction [89]. The role of LPD in RSA is disputed currently, and the endometrial biopsy is seldom applied in clinical practice.

11.2.11 Nightwork and Long Work Hours and RSA

Circadian rhythms are regulated by a "master clock" which located in the suprachiasmatic nucleus of the hypothalamus and are root in complex intracellular interactions, which referring to the so-called clock genes, which take part in feedback interactions that produce reproductive activity. Such clocks in the suprachiasmatic nucleus which received from the retina and transferred to the suprachiasmatic nucleus via the optic nerve (retinohypothalamic tract) stimulated by light-dark signals synchronously [90]. In human, the secretion of basal gonadotropin and LH surges exhibit diurnal rhythms. LH pulse frequency (and presumably GnRH) was influenced by sleep, and the effect of sleep can be regulated by developmental stage and sex steroid milieu [90].

Circadian clock genes were reported to express in the gravid uterus and placenta, where circadian controlled transcription and translation feedback loops are

applicable to this organ [91]. Almost every aspect of the immune response (innate and adaptive) was controlled by circadian oscillation [92]. Most immune cells such as macrophages, dendritic cells, T- and B-lymphocytes, impacts on host–pathogens interactions, leukocyte transport, activation, deactivation of innate and adaptive immunity responses exist in the circadian molecular clocks. When "rhythmic" perturbed, the role of immune cells can influence the maintain of the enriched vascular system which were needed for placentation and maternal-fetal immune tolerance [93].

The changes in demographic and epidemiologic profiles, eating habits, and job structures, with irregular working hours, particularly night shifts were changing features in the way of life in our society. Nightwork and long work hours induce the disturbance of sleepiness and dietary rhythm, result in deregulation of biological rhythms, which are related to fatigue, stress, lower performance in activities, greater risks of accidents, disruption of fertility [94, 95], and miscarriage [96–98].

Healthy sleep pattern leads to decreased daytime sleepiness, while long time work may induce abnormal sleep pattern. The sleepiness curves of workers who worked long hours appeared to be flat, poor quality, and lacking slow-wave or rapid eye movement ("deep") sleep. Circadian rhythm changes, whether due to sleep disturbances or melatonin production, may play a role in regulating reproductive hormones that control the menstrual cycle [99].

Research on working hours and menstrual status supports the impact of long hours (especially nightwork) on the menstrual cycle. More work hours per week and higher work intensity are associated with the prevalence of irregular menstruation and very short cycles, which may have implications for subfertility [100, 101]. Irregular menstrual cycles may affect fertility through the following mechanisms, early spontaneous abortion and increased risk of chronic diseases [102]. Firstly, the hypothalamic–pituitary axis is under the control of the circadian rhythm and affects ovulation time and gonadal hormone secretion [103]. In animal studies, it has been found that improper light exposure or physical activity to relieve circadian rhythm affects embryo implantation and successful pregnancy at the molecular level. If you rely on circadian rhythm, early reproductive results may be destroyed by shift work. Secondly, short or long menstrual cycles seem to be related to fertility and spontaneous abortion [102]. The short cycle may reflect a shortened luteal phase. Short luteal phase is associated with decreased progesterone [104], while low progesterone is associated with decreased pregnancy rate and increased abortion rate [105]. Decreased progesterone in the luteal phase is related to the different length of the follicular phase of the subsequent cycle, which may indicate that damage to the corpus luteum can cause gonadotropin secretion disorders [104]. Considering that follicular development and recruitment of dominant follicles occurred in the previous cycle, the decrease in fertility after a short cycle may be due to interference caused by poor oocyte quality. In previous studies of women who had repeated abortions, it was found that long periods are also related to spontaneous abortions [106]. The longer the cycle, the less developed follicles produce less estrogen. Low levels of estrogen in the follicular phase are associated with low fertility. In addition, low levels of estrogen may be accompanied by brief vaginal bleeding, which

indicates that the endometrium was underdeveloped in the previous cycle. If this trend is repeated in subsequent cycles, the endometrium may not be sufficient for implantation, resulting in low fertility or a high incidence of spontaneous abortion [102]. Thirdly, prolonged work and stress, mental or physical fatigue may lead to changes in circulating androgens and biochemical reactions (for example, increased secretion of cortisol and α-amylase), which may reduce fertility [107].

Insufficient sleep or poor quality after nightwork will also change the metabolism. Disturbance of the circadian rhythm caused by changes in the sleep-wake cycle caused by night work or continuous work will lead to alcoholic beverages, overweight, obesity, and unhealthy eating habits [108]. At the same time, the difficulty of participating in organized sports and leisure activities combined with fatigue may change behavior patterns and energy expenditure. This may be due to the fact that the lack of sleep common to nighttime workers exacerbates metabolic disorders such as glucose tolerance, insulin resistance, and dyslipidemia [109]. Human beings are naturally active individuals during the day. They should fast at night and endogenous glucose enters the bloodstream. Night shift workers usually suffer from loss of appetite, dyspepsia, and gastrointestinal diseases because many metabolic functions follow a pattern of circadian rhythms, including digestion, absorption, and nutrients [108].

In addition, changes in human lifestyles in recent decades have also affected eating habits. People who eat at night are more inclined to choose foods with high sugar and high fat taste [110]. They also prefer processed foods over foods rich in fiber and vitamins [111]. Eating snacks with high energy content but low nutritional value (low micronutrients and fiber content) during working hours has led to an increased incidence of overweight, obesity, and diabetes among these people [107, 112]. In addition, these foods may cause drowsiness due to hormones and neuroendocrine reactions caused by this nutrient, which is characterized by increased glucose, leptin, cholecystokinin, peptide YY, inflammatory cytokines, reduced norepinephrine, and reduced neurons wake-up signal [113]. These in turn can cause irregular menstruation, anovulation, and abortion.

11.2.12 Psychic Stress, Psychological Stress, and RSA

Psychological stress has been proposed to cause miscarriages [114, 115] and it has been reported that women with a history of psychological stress have almost twofold increase in miscarriage rate [115]. Psychological stress may include emotional trauma, social problems, concerns about money, uncoordinated marriage/partnership, work stress, major changes in personal circumstances, and previous pregnancy loss [116, 117].

Psychosocial stress has been shown to affect the nervous system, endocrine system, and immune system. The balance of these systems is severe for maintaining pregnancy. During exposure to pressure, the entire pressure regulating system, namely the hypothalamus–pituitary–adrenocortical system (HPA), various

hormones, including corticotropin-releasing hormone (CRH), corticotropin-releasing hormone (ACTH), cortex alcohol, and/or adrenaline are released into the blood in large amounts. Medium- [118] and long-term exposure to high levels of leather ketone will downregulate the glucocorticoid receptor, increase the level of folded corticosterone, and increase the response to stress, forming a vicious endocrine cycle. Instead, the glucocorticoid receptors of the dermal alcohol organs respond to both systemic stress and acoustic stimulation and play an important role in the mechanism of glucocorticoid receptor downregulation [119]. Stress-related early pregnancy failure may also be due to suppression of the hypothalamic–pituitary–gonadal axis [120].

Stress also reduces prolactin production in early pregnancy [121, 122]. Since prolactin stimulates the secretion of progesterone, lower prolactin levels will reduce the synthesis of progesterone [123, 124]. In addition, stress inhibits the secretion of pituitary gonadotropins, thereby inhibiting progesterone secretion by the corpus luteum [125, 126]. These mechanisms are related because the activity of progesterone is essential to maintain pregnancy. Low levels of progesterone in early pregnancy indicate miscarriage. In a variety of roles, this hormone helps to suppress the mother's immune response to pregnancy.

Under normal circumstances, after cellular stress or tissue damage, the molecules located inside the cell are usually released and bind to the pattern recognition receptor (PRR) on the surface of innate immune cells, thereby causing inflammation [127]. The mouse stress challenge model shows that by increasing mature uterine DC and reducing Treg cell drainage in the uterine lymph nodes and increasing the clonal expression of Th1 cells that secrete proinflammatory cytokines such as TNF-α and IFN-γ, Th2 cytokines (such as IL-10, IL-4, or TGF-β1) cannot fight, affecting fetal immune tolerance [128]. In turn, proinflammatory cytokines trigger the thrombotic inflammatory process in the maternal uterine placental blood vessels by releasing the procoagulant fgl2 prothrombin, increasing the frequency of vascular cell adhesion molecule 1 (VCAM-1) + blood vessels, thereby inducing embryonic and ischemic injury of the liver, resulting in miscarriage [129–131]. Socio-psychological stress can enhance gastrointestinal permeability and absorption of intestinal bacteria. LPS in the gastrointestinal tract becomes endogenous lipopolysaccharide through toll-like receptor 4 as a danger signal [132, 133]. NK cells act as sentinel cells, and environmental challenges can change their phenotype, for example, through epigenetic pathways, leading to reproductive failure [134].

Stress reduces the proliferation and efficacy of trophoblastic stem cells (TSC) and embryonic stem cells (ESC) and forces stem cells to differentiate to produce the minimum essential nutrient acquisition function mediated by the first differentiation lineage and then reduces cell growth. Stress reduces stem cell proliferation and anabolic metabolism without obvious apoptosis, but also reduces cell potential and increases cell differentiation to provide sufficient first lineage function [135].

Decreased sperm quality affects men's resistance to stress, which is related to reduced sperm concentration and early miscarriage [136]. In mice, psychological stress exposure may cause hypozincemia, which may be related to liver zinc accumulation, because glucocorticoid-mediated MT synthesis and interleukin

6-induced ZIP14 expression high levels of MT [137]. In addition, after repeated psychological stress exposure, serum iron levels in the villi intestinal cells, liver, spleen, cerebral cortex, hippocampus, and striatum of rats decreased, and iron accumulated significantly [138, 139]. PS exposure enhances the oxidative response in rat brain and plasma [138, 140].

Chronic stress exposure may result in increased expression of the hypothalamic inhibitory peptide RFamide-related peptide 3 (RFRP3) in women who regularly cycle even after quitting smoking, which in turn leads to persistent maladaptive sexual dysfunction, decreased fertility, and frequent pregnancy recurrent embryo loss [141]. Glucocorticoid levels may mediate this effect, because two glucocorticoid response elements (GREs) are found in the RFRP promoter region, which means that RFRP may be directly regulated by circulating glucocorticoid levels [142]. Using inducible targeted shRNA, RFRP3 gene silencing during stress can completely relieve stress-induced infertility in female rats, resulting in a difference in mating and pregnancy success rates from the non-stress control group [143].

The experience of miscarriage may in turn increase the level of distress, anxiety, and depression, and the effects of miscarriage may be lost during pregnancy until the next pregnancy [144–147]. Women who are pregnant after a perinatal abortion have a higher level of depression [148, 149], anxiety, and depression, which may continue until 33 months postpartum [150]. The link between psychological stress and miscarriage may be partly due to the activation of the hypothalamic–pituitary–adrenal axis by hypothalamic neurons that secrete corticotropin-releasing hormone, increasing the secretion of adrenocortical nutrients and adrenocortical hormone secretion by the pituitary [120]. This hormone has a direct effect on the metabolism of the decidua and placenta, but it also interacts with progesterone signaling [121]. Stress-related early pregnancy failure may also be due to suppression of the hypothalamic–pituitary–gonadal axis [120].

11.2.13 Medical or Surgical Abortion and RSA

In 1997, the World Health Organization has reported that 53 million unplanned pregnancies result in artificial termination each year [151]. Nowadays, approximately 15–20% of all clinically confirmed pregnancies end in a miscarriage [152]. Intentional termination of pregnancy before viability of the fetus is defined as induced abortion [153]. At 9 weeks of gestation or less, medical and surgical abortion has a similar efficacy with about greater than 99% success rates [154, 155]. There are still known risks and adverse effects that more attention must be paid. Potential complications related to abortions including pain, bleeding, an incomplete abortion which had to proceed with a vacuum curettage, or an infection in the upper genital tract that causes endometritis, oophoritis, and salpingitis [156, 157]. Unsafe abortion is associated with maternal morbidities and mortality. An estimated 289,000 maternal death happened in 2013. The global maternal mortality rate (MMR) was 210 maternal deaths per 100,000 live births in 2013.

Moreover, the MMR in developing regions was 14 times higher than that in developed regions [158]. Genital tract injuries, vesicovaginal fistula, gastrointestinal injuries, acute renal failure, uterine perforation, septicemia, and infertility are all complications of abortion [159].

Surgical abortion (SA) in the first trimester may not increase the risk of adverse outcome in subsequent pregnancies [160, 161]. Rates of depression are not significantly different between women obtaining abortion and those denied abortion [162]. As medical abortion is convenient, inexpensive, and noninvasive, many women desiring to choose it to terminate a pregnancy [163]. Studies also showed that women who underwent SA were more likely to have miscarriages in the next pregnancy [164], which may associate with cervical injury, postabortal infection, or intrauterine adhesions (IUA).

If women undergo surgical abortion, it may lead to cervical injury, especially undergoing surgical cervical dilatation. Operation gently is recommended when cervical dilatation was done. Infection and IUA are the common cause of recurrent spontaneous abortion as well as uterine infertility. The incidence of postabortal infections varies between studies. The frequency of infectious complications from medical abortions was about 2.4–4.8% and 4.9% from surgical abortions [165, 166]. Infections related to abortions are often by chlamydia, gonorrhea, mycoplasma, and bacterial vaginosis (BV) that proceeds from the lower genitals and moves through the cervix to the uterus [167]. The occurrence of endometritis accompanied with increased expression of IL-17, IL-6, IL-1beta, TNF-alpha decreased expression of IL-10 and TGF-β [168, 169]. The imbalanced expression of Th1, Th2, Th17 of endometritis is associated with recurrent spontaneous abortion [169, 170]. The untreated infection can spread to the fallopian tubes and then lead to miscarriage. Antibiotic treatment is given if/when a bacterial infection is identified, but the timing of the antibiotic administration does not affect the rate of postabortal infection [156, 166, 171].

Severe damage to the endometrium leads to endometrium fibrotic regeneration than IUA [152, 172]. About one in five women develop scarring after a miscarriage [152]. Less often, intrauterine adhesions result from an infection, such as genital tuberculosis [173]. Prevention of reformation of adhesions is still the challenging problem and no single method for preventing recurrence has shown superiority. Hysteroscopic lysis of adhesions is now the gold standard for treatment. Estrogen with moderate dosage may inhibit endometrium fibrosis and improve endometrium receptivity [174]. IUD is beneficial in patients with different degrees of IUA, still needs to be combined with other ancillary treatments to obtain maximal outcomes [175]. The use of an amnion graft after intrauterine adhesiolysis appears to be beneficial in reducing the recurrence of adhesion and reformation improving menstruation [176]. Endometrial stem/progenitor cells may play a role in regenerating inadequate endometrium [173]. Bone marrow-derived mesenchymal stem cells (BMSCs) transplantation could promote the expression of ER and PR,was effective to repair the damaged endometrium [177].

11.2.14 Microelements and RSA

Microelements or trace elements are iron (Fe), calcium, manganese (Mn), zinc (Zn), boron (B), important indicator of maternal nutritional status which is associated with fetal growth and development during pregnancy. They play a role in normal metabolism as well as immune function during pregnancy [178]. Excess or deficiency of microelements is associated with miscarriage and other adverse pregnancy outcomes [179].

Lower Cu [179–182] as well as higher Cu [183, 184] concentrations were found in pathological conditions, such as spontaneous abortion, threatened abortion, missed abortion, blighted ovum, and intrauterine growth restriction. The majority of copper exists as ceruloplasmin and their function is to protect cells from the toxic superoxide anion, maintaining cell proliferation, normal hematopoietic function, and immune function [181, 185]. Progesterone and estrogen stimulate the liver to increase synthesis of ceruloplasmin, and the abnormal Cu level may collate with placental insufficiency [186].

Zinc is a component of a variety of enzymes and nucleic acid [187, 188], plays an important role in the human immune system and the development of the fetal nervous system [189, 190]. Decreased zinc was found in spontaneous abortion [184, 191]. Zn, Se, Mn, and Cu are also ingredients of enzymes in the first line of defense taking part in expelling free radicals. Low level of Zn but high of Mn may be indicative of the incidence of miscarriage [192]. Decline serum concentration of Zn and raise serum concentration of Cu was found under inflammatory conditions, Cu/Zn ratio might be associated with the occurrence of spontaneous abortion [193, 194].

Calcium plays an important role in the activation of muscle-keeping, nervous excitement, and enzyme activation. Maternal serum calcium increases the risk of miscarriage [195–199], and approximately 72% of all pregnancy losses occurred in women with a serum calcium level of 11·4 mg/dl or higher [200]. Decrease in magnesium was found in recurrent spontaneous abortions [201, 202]. High Ca^{2+} level may mediate the dysfunction of trophoblast infiltration, result in trophoblast apoptosis, which leads to recurrent spontaneous abortion [203].

Iron is involved in oxygen transport, storage, and use, the synthesis of cytochrome enzymes, peroxidase enzymes, and hormones [204]. Iron deficiency can cause chronic lack of oxygen in mother and fetus [205–207]. The metabolism of copper and iron is tightly interlinked during pregnancy. Iron deficiency in the mother results in an increase in liver copper levels which may result in spontaneous abortion [208].

Selenium is an essential trace element important for the functions of immune and reproductive systems, metabolism of thyroid hormones, as well as antioxidant defense. Increased incidence of spontaneous abortion collated with selenium deficiency [209–212].

In conclusion, determination of the trace elements in plasma of patients with spontaneous abortion may be benefit in the prevention of miscarriages.

References

1. Rpl EGGO, Bender Atik R, Christiansen OB, et al. Eshre guideline: recurrent pregnancy loss. Hum Reprod Open. 2018;2018(2):Hoy004.
2. Practice Committee of the American Society for Reproductive. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98(5):1103–11.
3. Medicine P C O T A S F R. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98:5.
4. Youssef A, Vermeulen N, Lashley E, et al. Comparison And appraisal of (inter)national recurrent pregnancy loss guidelines. Reprod Biomed Online. 2019;39(3):497–503.
5. Practice Committee of the American Society for Reproductive. Effectiveness and treatment for unexplained infertility. Fertil Steril. 2006;86(5 Suppl 1):S111–4.
6. Bernicot I, Schneider A, Mace A, et al. Analysis using fish of sperm and embryos from two carriers of rare rob(13;21) and rob(15;22) robertsonian translocation undergoing PGD. Eur J Med Genet. 2012;55(4):245–51.
7. Iravani AT, Saeedi MM, Pakravesh J, et al. Thyroid autoimmunity and recurrent spontaneous abortion in Iran: a case-control study. Endocr Pract. 2008;14(4):458–64.
8. Giakoumelou S, Wheelhouse N, Cuschieri K, et al. The role of infection in miscarriage. Hum Reprod Update. 2016;22(1):116–33.
9. Rein DT, Schmidt T, Hess AP, et al. Hysteroscopic management of residual trophoblastic tissue is superior to ultrasound-guided curettage. J Minim Invasive Gynecol. 2011;18 (6):774–8.
10. Kokcu A, Yavuz E, Celik H, et al. A panoramic view to relationships between reproductive failure and immunological factors. Arch Gynecol Obstet. 2012;286(5):1283–9.
11. Hart K, Tadros NN. The role of environmental factors and lifestyle on male reproductive health, the epigenome, and resulting offspring. Panminerva Med. 2019;61(2):187–95.
12. Epstein SS. Environmental pathology. A review. Am J Pathol. 1972;66(2):352–74.
13. Manassaram DM, Backer LC, Moll DM. A review of nitrates in drinking water: maternal exposure and adverse reproductive and developmental outcomes. Environ Health Perspect. 2006;114(3):320–7.
14. Mcdiarmid MA, Gardiner PM, Jack BW. The clinical content of preconception care: environmental exposures. Am J Obstet Gynecol. 2008;199(6 Suppl 2):S357–61.
15. Neuman G, Gareri J, Koren G. Preconceptional monitoring of mercury levels in hair and blood as a tool for minimizing associated reproductive risks. Ther Drug Monit. 2014;36(6):696–8.
16. James LF, Panter KE, Nielsen DB, et al. The effect of natural toxins on reproduction in livestock. J Anim Sci. 1992;70(5):1573–9.
17. Ray AC, Abbitt B, Cotter SR, et al. Bovine abortion and death associated with consumption of aflatoxin-contaminated peanuts. J Am Vet Med Assoc. 1986;188(10):1187–8.
18. Ribelin WE, Fukushima K, Still PE. The toxicity of ochratoxin to ruminants. Can J Comp Med. 1978;42(2):172–6.
19. Riet-Correa F, Medeiros RM, Schild AL. A review of poisonous plants that cause reproductive failure and malformations in the ruminants of Brazil. J Appl Toxicol. 2012;32(4):245–54.
20. Panter KE, James LF. Natural plant toxicants in milk: a review. J Anim Sci. 1990;68 (3):892–904.
21. Molyneux RJ, James LF. Pyrrolizidine alkaloids in milk: thresholds of intoxication. Vet Hum Toxicol. 1990;32(Suppl):94–103.
22. Becker-Algeri TA, Castagnaro D, De Bortoli K, et al. Mycotoxins in bovine milk and dairy products: a review. J Food Sci. 2016;81(3):R544–52.
23. James LF, Hartley WJ. Effects of milk from animals fed locoweed on kittens, calves, and lambs. Am J Vet Res. 1977;38(8):1263–5.
24. Mendola P, Messer LC, Rappazzo K. Science linking environmental contaminant exposures with fertility and reproductive health impacts in the adult female. Fertil Steril. 2008;89 (2 Suppl):E81–94.

25. Velez MP, Arbuckle TE, Fraser WD. Maternal exposure to perfluorinated chemicals and reduced fecundity: the mirec study. Hum Reprod. 2015;30(3):701–9.
26. Gerhard I, Daniel V, Link S, et al. Chlorinated hydrocarbons in women with repeated miscarriages. Environ Health Perspect. 1998;106(10):675–81.
27. Shen S, Yuan L, Zeng S. An effort to test the embryotoxicity of benzene, toluene, xylene, and formaldehyde to murine embryonic stem cells using airborne exposure technique. Inhal Toxicol. 2009;21(12):973–8.
28. Sallmen M, Lindbohm ML, Kyyronen P, et al. Reduced fertility among women exposed to organic solvents. Am J Ind Med. 1995;27(5):699–713.
29. Taskinen H, Kyyronen P, Hemminki K, et al. Laboratory work and pregnancy outcome. J Occup Med. 1994;36(3):311–9.
30. Kyyronen P, Taskinen H, Lindbohm ML, et al. Spontaneous abortions and congenital malformations among women exposed to tetrachloroethylene in dry cleaning. J Epidemiol Community Health. 1989;43(4):346–51.
31. Rahman A, Kumarathasan P, Gomes J. Infant and mother related outcomes from exposure to metals with endocrine disrupting properties during pregnancy. Sci Total Environ. 2016;569-570:1022–31.
32. Arbuckle TE, Lin Z, Mery LS. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environ Health Perspect. 2001;109(8):851–7.
33. Arbuckle TE, Savitz DA, Mery LS, et al. Exposure to phenoxy herbicides and the risk of spontaneous abortion. Epidemiology. 1999;10(6):752–60.
34. Hakeem O, Jabri S. Adverse birth outcomes in women exposed to Syrian chemical attack. Lancet Glob Health. 2015;3(4):E196.
35. Yanai J, Pinkas A, Seidler FJ, et al. Neurobehavioral teratogenicity of Sarin in an avian model. Neurotoxicol Teratol. 2009;31(6):406–12.
36. Groen RS, Bae JY, Lim KJ. Fear of the unknown: ionizing radiation exposure during pregnancy. Am J Obstet Gynecol. 2012;206(6):456–62.
37. Homan GF, Davies M, Norman R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. Hum Reprod Update. 2007;13(3):209–23.
38. Ozbayrak M, Cavdar I, Seven M, et al. Determining and managing fetal radiation dose from diagnostic radiology procedures in Turkey. Korean J Radiol. 2015;16(6):1276–82.
39. Roux C, Horvath C, Dupuis R. Effects of pre-implantation low-dose radiation on rat embryos. Health Phys. 1983;45(5):993–9.
40. Committee Opinion No. 723. Guidelines for diagnostic imaging during pregnancy and lactation. Obstet Gynecol. 2017;130(4):E210–E6.
41. ICRP. Pregnancy and medical radiation. Ann ICRP. 2000;30(1):1–43.
42. Brent RL. Protection of the gametes embryo/fetus from prenatal radiation exposure. Health Phys. 2015;108(2):242–74.
43. Brent RL. Saving lives and changing family histories: appropriate counseling of pregnant women and men and women of reproductive age, concerning the risk of diagnostic radiation exposures during and before pregnancy. Am J Obstet Gynecol. 2009;200(1):4–24.
44. Donnelly EH, Smith JM, Farfan EB, et al. Prenatal radiation exposure: background material for counseling pregnant patients following exposure to radiation. Disaster Med Public Health Prep. 2011;5(1):62–8.
45. Perko T, Raskob W, Jourdain JR. Improved communication, understanding of risk perception and ethics related to ionising radiation. J Radiol Prot. 2016;36(2):E15–22.
46. Doyle P, Maconochie N, Roman E, et al. Fetal death and congenital malformation in babies born to nuclear industry employees: report from the nuclear industry family study. Lancet. 2000;356(9238):1293–9.
47. Lassi ZS, Imam AM, Dean SV, et al. Preconception care: caffeine, smoking, alcohol, drugs and other environmental chemical/radiation exposure. Reprod Health. 2014;11(Suppl 3):S6.

48. Ouellet-Hellstrom R, Stewart WF. Miscarriages among female physical therapists who report using radio- and microwave-frequency electromagnetic radiation. Am J Epidemiol. 1993;138 (10):775–86.
49. Shah SG, Farrow A. Systematic literature review of adverse reproductive outcomes associated with physiotherapists' occupational exposures to non-ionising radiation. J Occup Health. 2014;56(5):323–31.
50. Wieseler KM, Bhargava P, Kanal KM, et al. Imaging In pregnant patients: examination appropriateness. Radiographics. 2010;30(5):1215–29.
51. Colletti PM, Lee KH, Elkayam U. Cardiovascular imaging of the pregnant patient. AJR. 2013;200(3):515–21.
52. Guilbaud L, Beghin D, Dhombres F, et al. Pregnancy outcome after first trimester exposure to ionizing radiations. Eur J Obstet Gynecol Reprod Biol. 2019;232:18–21.
53. Guan L, Chen G, Zhang J, et al. The preliminary clinical observation and analysis of childbearing age women with a history of iodine-131 treatment for graves' disease. Biosci Trends. 2016;10(4):307–14.
54. Chiarelli AM, Marrett LD, Darlington GA. Pregnancy outcomes in females after treatment for childhood cancer. Epidemiology. 2000;11(2):161–6.
55. Winther JF, Boice JD Jr, Svendsen AL, et al. Spontaneous abortion in a Danish population-based cohort of childhood cancer survivors. J Clin Oncol. 2008;26(26):4340–6.
56. Gao W, Liang JX, Yan Q. Exposure to radiation therapy is associated with female reproductive health among childhood cancer survivors: a meta-analysis study. J Assist Reprod Genet. 2015;32(8):1179–86.
57. Heidecker B, Spencer RM, Hayes V, et al. High prevalence and clinical/sociodemographic correlates of miscarriages among flight attendants. Am J Med. 2017;130(12):1397–401.
58. Grajewski B, Whelan EA, Lawson CC, et al. Miscarriage among flight attendants. Epidemiology. 2015;26(2):192–203.
59. Chen H, Qu Z, Liu W. Effects of simulated mobile phone electromagnetic radiation on fertilization and embryo development. Fetal Pediatr Pathol. 2017;36(2):123–9.
60. Megaw L, Clemens T, Dibben C, et al. Pregnancy outcome and ultraviolet radiation; a systematic review. Environ Res. 2017;155:335–43.
61. Judistiani RTD, Nirmala SA, Rahmawati M, et al. Optimizing ultraviolet B radiation exposure to prevent vitamin D deficiency among pregnant women in the tropical zone: report from cohort study on vitamin D status and its impact during pregnancy in Indonesia. BMC Pregnancy Childbirth. 2019;19(1):209.
62. Kwak-Kim J, Skariah A, Wu L, et al. Humoral and cellular autoimmunity in women with recurrent pregnancy losses and repeated implantation failures: a possible role of vitamin D. Autoimmun Rev. 2016;15(10):943–7.
63. Zeng M, Su S, Li L. The effect of laser-assisted hatching on pregnancy outcomes of cryopreserved-thawed embryo transfer: a meta-analysis of randomized controlled trials. Lasers Med Sci. 2018;33(3):655–66.
64. Jauchem JR. Effects of low-level radio-frequency (3 kHz to 300 GHz) energy on human cardiovascular, reproductive, immune, and other systems: a review of the recent literature. Int J Hyg Environ Health. 2008;211(1-2):1–29.
65. Qu XL, Wang HT, Zou JL, et al. Effect of transvaginal ultrasound on human chorionic villus cell apoptosis during pregnancy. Genet Mol Res. 2015;14(4):18771–7.
66. Arck PC, Merali FS, Manuel J, et al. Stress-triggered abortion: inhibition of protective suppression and promotion of tumor necrosis factor-alpha (TNF-alpha) release as a mechanism triggering resorptions in mice. Am J Reprod Immunol. 1995;33(1):74–80.
67. Blois S, Tometten M, Kandil J, et al. Intercellular adhesion molecule-1/Lfa-1 cross talk is a proximate mediator capable of disrupting immune integration and tolerance mechanism at the feto-maternal interface in murine pregnancies. J Immunol. 2005;174(4):1820–9.

68. Demko ZP, Simon AL, McCoy RC, Petrov DA, Rabinowitz M. Effects of maternal age on euploidy rates in a large cohort of embryos analyzed with 24-chromosome single-nucleotide polymorphism-based preimplantation genetic screening. Fertil Steril. 2016;105(5):1307–13.
69. Kovalevsky G, Gracia CR, Berlin JA, et al. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. Arch Intern Med. 2004;164 (5):558–63.
70. Onderoglu L, Baykal C, Al RA, et al. High frequency of thrombophilic disorders in women with recurrent fetal miscarriage. Clin Exp Obstet Gynecol. 2006;33(1):50–4.
71. Loew A, Jacob D, Neuhaus P, et al. Resistance to activated protein c caused by factor V Leiden mutation and orthotopic liver transplantation. Transplantation. 2005;79(10):1422–7.
72. Kurzawinska G, Seremak-Mrozikiewicz A, Drews K, et al. Inherited thrombophilia as the reason of recurrent miscarriages in the first trimester of pregnancy. Ginekol Pol. 2009;80 (9):657–63.
73. Rai R, Backos M, Rushworth F, et al. Polycystic ovaries and recurrent miscarriage--a reappraisal. Hum Reprod. 2000;15(3):612–5.
74. Wang JX, Davies MJ, Norman RJ. Obesity increases the risk of spontaneous abortion during infertility treatment. Obes Res. 2002;10(6):551–4.
75. Tian L, Shen H, Lu Q, et al. Insulin resistance increases the risk of spontaneous abortion after assisted reproduction technology treatment. J Clin Endocrinol Metab. 2007;92(4):1430–3.
76. Maryam K, Bouzari Z, Basirat Z, et al. The comparison of insulin resistance frequency in patients with recurrent early pregnancy loss to normal individuals. BMC Res Notes. 2012;5:133.
77. Schachter M, Raziel A, Friedler S, et al. Insulin resistance in patients with polycystic ovary syndrome is associated with elevated plasma homocysteine. Hum Reprod. 2003;18(4):721–7.
78. Wijeyaratne CN, Nirantharakumar K, Balen AH, et al. Plasma homocysteine in polycystic ovary syndrome: does it correlate with insulin resistance and ethnicity? Clin Endocrinol. 2004;60(5):560–7.
79. Sun L, Lv H, Wei W, et al. Angiotensin-converting enzyme D/I and plasminogen activator inhibitor-1 4g/5g gene polymorphisms are associated with increased risk of spontaneous abortions in polycystic ovarian syndrome. J Endocrinol Investig. 2010;33(2):77–82.
80. Gosman GG, Katcher HI, Legro RS. Obesity and the role of gut and adipose hormones in female reproduction. Hum Reprod Update. 2006;12(5):585–601.
81. Gabbe SG, Graves CR. Management of diabetes mellitus complicating pregnancy. Obstet Gynecol. 2003;102(4):857–68.
82. Gutaj P, Zawiejska A, Wender-Ozegowska E, et al. Maternal factors predictive of first trimester pregnancy loss in women with pregestational diabetes. Pol Arch Med. 2013;123 (1-2):21–8.
83. Glinoer D. Thyroid hyperfunction during pregnancy. Thyroid. 1998;8(9):859–64.
84. Millar LK, Wing DA, Leung AS, et al. Low birth weight and preeclampsia in pregnancies complicated by hyperthyroidism. Obstet Gynecol. 1994;84(6):946–9.
85. Kriplani A, Buckshee K, Bhargava VL, et al. Maternal and perinatal outcome in thyrotoxicosis complicating pregnancy. Eur J Obstet Gynecol Reprod Biol. 1994;54(3):159–63.
86. Momotani N, Noh J, Oyanagi H, et al. Antithyroid drug therapy for Graves' disease during pregnancy. Optimal regimen for fetal thyroid status. N Engl J Med. 1986;315(1):24–8.
87. Allan WC, Haddow JE, Palomaki GE, et al. Maternal thyroid deficiency and pregnancy complications: implications for population screening. J Med Screen. 2000;7(3):127–30.
88. Arredondo F, Noble LS. Endocrinology of recurrent pregnancy loss. Semin Reprod Med. 2006;24(1):33–9.
89. Insler V. Corpus luteum defects. Curr Opin Obstet Gynecol. 1992;4(2):203–11.
90. Jerome F, Yen S, Jaffe I. Reproductive endocrinology: physiology, pathophysiology, and clinical management. 7th ed. London: Elsevier; 2014.
91. Waddell BJ, Wharfe MD, Crew RC, et al. A rhythmic placenta? Circadian variation, clock genes and placental function. Placenta. 2012;33(7):533–9.

92. Silver AC, Arjona A, Walker WE, et al. The Circadian clock controls toll-like receptor 9-mediated innate and adaptive immunity. Immunity. 2012;36(2):251–61.
93. Man GCW, Zhang T, Chen X, et al. The regulations and role of circadian clock and melatonin in uterine receptivity and pregnancy-an immunological perspective. Am J Reprod Immunol. 2017;78:2.
94. James SM, Honn KA, Gaddameedhi S, et al. Shift work: disrupted circadian rhythms and sleep-implications for health and well-being. Curr Sleep Med Rep. 2017;3(2):104–12.
95. Kecklund G, Axelsson J. Health consequences of shift work and insufficient sleep. BMJ. 2016;355:5210.
96. Bonde JP, Jorgensen KT, Bonzini M, et al. Miscarriage and occupational activity: a systematic review and meta-analysis regarding shift work, working hours, lifting, standing, and physical workload. Scand J Work Environ Health. 2013;39(4):325–34.
97. Stocker LJ, Macklon NS, Cheong YC, et al. Influence of shift work on early reproductive outcomes: a systematic review and meta-analysis. Obstet Gynecol. 2014;124(1):99–110.
98. Feodor Nilsson S, Andersen PK, Strandberg-Larsen K, et al. Risk factors for miscarriage from a prevention perspective: a nationwide follow-up study. BJOG. 2014;121(11):1375–84.
99. Martins AJ, Martini LA, Moreno CRC, Prudent Diet I. Associated with low sleepiness among short-haul truck drivers. Nutrition. 2018;63-64:61–8.
100. Lawson CC, Johnson CY, Chavarro JE, et al. Work schedule and physically demanding work in relation to menstrual function: the nurses' health study 3. Scand J Work Environ Health. 2015;41(2):194–203.
101. Axmon A, Rylander L, Albin M, et al. Factors affecting time to pregnancy. Hum Reprod. 2006;21(5):1279–84.
102. Small CM, Manatunga AK, Klein M, et al. Menstrual cycle characteristics: associations with fertility and spontaneous abortion. Epidemiology. 2006;17(1):52–60.
103. Sellix MT, Menaker M. Circadian clocks in mammalian reproductive physiology: effects of the "other" biological clock on fertility. Discov Med. 2011;11(59):273–81.
104. Windham GC, Elkin E, Fenster L, et al. Ovarian hormones in premenopausal women: variation by demographic, reproductive and menstrual cycle characteristics. Epidemiology. 2002;13(6):675–84.
105. Wuttke W, Pitzel L, Seidlova-Wuttke D, et al. LH pulses and the corpus luteum: the luteal phase deficiency LPD. Vitam Horm. 2001;63:131–58.
106. Quenby SM, Farquharson RG. Predicting recurring miscarriage: what is important? Obstet Gynecol. 1993;82(1):132–8.
107. Ulhoa MA, Marqueze EC, Burgos LG, et al. Shift work and endocrine disorders. Int J Endocrinol. 2015;2015:826249.
108. Lowden A, Moreno C, Holmback U, et al. Eating and shift work - effects on habits, metabolism and performance. Scand J Work Environ Health. 2010;36(2):150–62.
109. Depner CM, Stothard ER, Wright KP Jr. Metabolic consequences of sleep and circadian disorders. Curr Diab Rep. 2014;14(7):507.
110. Gallant A, Drapeau V, Allison KC, et al. Night eating behavior and metabolic heath in mothers and fathers enrolled in the quality cohort study. Eat Behav. 2014;15(2):186–91.
111. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. Nutr Rev. 2012;70(1):3–21.
112. Balieiro LC, Rossato LT, Waterhouse J, et al. Nutritional status and eating habits of bus drivers during the day and night. Chronobiol Int. 2014;31(10):1123–9.
113. Panossian LA, Veasey SC. Daytime sleepiness in obesity: mechanisms beyond obstructive sleep apnea--a review. Sleep. 2012;35(5):605–15.
114. Kwak-Kim J, Bao S, Lee SK, et al. Immunological modes of pregnancy loss: inflammation, immune effectors, and stress. Am J Reprod Immunol. 2014;72(2):129–40.
115. Qu F, Wu Y, Zhu YH, et al. The association between psychological stress and miscarriage: a systematic review and meta-analysis. Sci Rep. 2017;7(1):1731.

116. Brandt LP, Nielsen CV. Job stress and adverse outcome of pregnancy: a causal link or recall bias? Am J Epidemiol. 1992;135(3):302–11.
117. Kicia M, Skurzak A, Wiktor K, Iwanowicz-Palus G, Wiktor H. Anxiety and stress in miscarriage. Pol J Publ Health. 2015;125:162–5.
118. Mulder EJ, Robles De Medina PG, Huizink AC, et al. Prenatal maternal stress: effects on pregnancy and the (unborn) child. Early Hum Dev. 2002;70(1-2):3–14.
119. Hebert S, Paiement P, Lupien SJ. A physiological correlate for the intolerance to both internal and external sounds. Hear Res. 2004;190(1-2):1–9.
120. Stergiakouli E, Sterne JA, Smith GD. Failure to replicate the association of glucocorticoid and type 1 corticotropin-releasing hormone receptors gene variants with risk of depression during pregnancy and post-partum reported by. J Psychiatr Res. 2014;56:168–70.
121. Parker VJ, Douglas AJ. Stress in early pregnancy: maternal neuro-endocrine-immune responses and effects. J Reprod Immunol. 2010;85(1):86–92.
122. Labad J, Stojanovic-Perez A, Montalvo I, et al. Stress biomarkers as predictors of transition to psychosis in at-risk mental states: roles for cortisol, prolactin and albumin. J Psychiatr Res. 2015;60:163–9.
123. Pennacchio GE, Neira FJ, Soaje M, et al. Effect of hyperthyroidism on circulating prolactin and hypothalamic expression of tyrosine hydroxylase, prolactin signaling cascade members and estrogen and progesterone receptors during late pregnancy and lactation in the rat. Mol Cell Endocrinol. 2017;442:40–50.
124. Varas SM, Jahn GA. The expression of estrogen, prolactin, and progesterone receptors in mammary gland and liver of female rats during pregnancy and early postpartum: regulation by thyroid hormones. Endocr Res. 2005;31(4):357–70.
125. Surico D, Farruggio S, Marotta P, et al. Human chorionic gonadotropin protects vascular endothelial cells from oxidative stress by apoptosis inhibition, cell survival signalling activation and mitochondrial function protection. Cell Physiol Biochem. 2015;36(6):2108–20.
126. Kajihara T, Uchino S, Suzuki M, et al. Human chorionic gonadotropin confers resistance to oxidative stress-induced apoptosis in decidualizing human endometrial stromal cells. Fertil Steril. 2011;95(4):1302–7.
127. Seong SY, Matzinger P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. Nat Rev Immunol. 2004;4(6):469–78.
128. Blois SM, Kammerer U, Alba Soto C, et al. Dendritic cells: key to fetal tolerance? Biol Reprod. 2007;77(4):590–8.
129. Clark DA, Ding JW, Yu G, et al. Fgl2 prothrombinase expression in mouse trophoblast and decidua triggers abortion but may be countered by Ox-2. Mol Hum Reprod. 2001;7 (2):185–94.
130. Clark DA, Blois S, Kandil J, et al. Reduced uterine indoleamine 2,3-dioxygenase versus increased Th1/Th2 cytokine ratios as a basis for occult and clinical pregnancy failure in mice and humans. Am J Reprod Immunol. 2005;54(4):203–16.
131. Prados MB, Solano ME, Friebe A, et al. Stress increases Vcam-1 expression at the fetomaternal interface in an abortion-prone mouse model. J Reprod Immunol. 2011;89 (2):207–11.
132. Friebe A, Arck P. Causes for spontaneous abortion: what the bugs 'gut' to do with it? Int J Biochem Cell Biol. 2008;40(11):2348–52.
133. Friebe A, Douglas AJ, Solano E, et al. Neutralization of LPS Or blockage of TLR4 signaling prevents stress-triggered fetal loss in murine pregnancy. J Mol Med. 2011;89(7):689–99.
134. Karimi K, Arck PC. Natural killer cells: keepers of pregnancy in the turnstile of the environment. Brain Behav Immun. 2010;24(3):339–47.
135. Yang Y, Bolnick A, Shamir A, et al. Blastocyst-derived stem cell populations under stress: impact of nutrition and metabolism on stem cell potency loss and miscarriage. Stem Cell Rev. 2017;13(4):454–64.
136. Zorn B, Auger J, Velikonja V, et al. Psychological factors in male partners of infertile couples: relationship with semen quality and early miscarriage. Int J Androl. 2008;31(6):557–64.

137. Tian X, Zheng Y, Li Y, et al. Psychological stress induced zinc accumulation and up-regulation of Zip14 and metallothionein in rat liver. BMC Gastroenterol. 2014;14:32.
138. Wang L, Wang W, Zhao M, et al. Psychological stress induces dysregulation of iron metabolism in rat brain. Neuroscience. 2008;155(1):24–30.
139. Zhao M, Chen J, Wang W, et al. Psychological stress induces hypoferremia through the Il-6-hepcidin axis in rats. Biochem Biophys Res Commun. 2008;373(1):90–3.
140. Scarpellini F, Sbracia M, Scarpellini L. Psychological stress and lipoperoxidation in miscarriage. Ann N Y Acad Sci. 1994;709:210–3.
141. Young AJ, Carlson AA, Monfort SL, et al. Stress and the suppression of subordinate reproduction in cooperatively breeding Meerkats. Proc Natl Acad Sci U S A. 2006;103 (32):12005–10.
142. Son YL, Ubuka T, Narihiro M, et al. Molecular Basis for the Activation of Gonadotropin-Inhibitory Hormone Gene Transcription by Corticosterone. Endocrinology. 2014;155 (5):1817–26.
143. Geraghty AC, Muroy SE, Zhao S, et al. Knockdown of hypothalamic Rfrp3 prevents chronic stress-induced infertility and embryo resorption. elife. 2015;4:e04316.
144. San Lazaro Campillo I, Meaney S, McNamara K, et al. Psychological and support interventions to reduce levels of stress, anxiety or depression on women's subsequent pregnancy with a history of miscarriage: an empty systematic review. BMJ Open. 2017;7(9):E017802.
145. Krosch DJ, Shakespeare-Finch J. Grief, traumatic stress, and posttraumatic growth in women who have experienced pregnancy loss. Psychol Trauma. 2017;9(4):425–33.
146. Tavoli Z, Mohammadi M, Tavoli A, et al. Quality of life and psychological distress in women with recurrent miscarriage: a comparative study. Health Qual Life Outcomes. 2018;16(1):150.
147. Mccarthy FP, Moss-Morris R, Khashan AS, et al. Previous pregnancy loss has an adverse impact on distress and behaviour in subsequent pregnancy. BJOG. 2015;122(13):1757–64.
148. Gong X, Hao J, Tao F, et al. Pregnancy loss and anxiety and depression during subsequent pregnancies: data from the C-Abc study. Eur J Obstet Gynecol Reprod Biol. 2013;166 (1):30–6.
149. Bicking Kinsey C, Baptiste-Roberts K, Zhu J, et al. Effect of previous miscarriage on depressive symptoms during subsequent pregnancy and postpartum in the first baby study. Matern Child Health J. 2015;19(2):391–400.
150. Blackmore ER, Cote-Arsenault D, Tang W, et al. Previous prenatal loss as a predictor of perinatal depression and anxiety. Br J Psychiatry. 2011;198(5):373–8.
151. Medical Methods For Termination Of Pregnancy. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser. 1997;871:1–110.
152. Hooker AB, Lemmers M, Thurkow AL, et al. Systematic review and meta-analysis of intrauterine adhesions after miscarriage: prevalence, risk factors and long-term reproductive outcome. Hum Reprod Update. 2014;20(2):262–78.
153. Cunnigham LK, Bloom S, Hauth J, Rouse D, Spong C. Williams obstetrics. 23rd ed. New York: McGraw-Hill; 2010.
154. Ireland LD, Gatter M, Chen AY. Medical compared with surgical abortion for effective pregnancy termination in the first trimester. Obstet Gynecol. 2015;126(1):22–8.
155. Mcgregor JA, Equiles O. Risks of mifepristone abortion in context. Contraception. 2005;72 (5):393.
156. Carlsson I, Breding K, Larsson PG. Complications related to induced abortion: a combined retrospective and longitudinal follow-up study. BMC Womens Health. 2018;18(1):158.
157. Niinimaki M, Suhonen S, Mentula M, et al. Comparison of rates of adverse events in adolescent and adult women undergoing medical abortion: population register based study. BMJ. 2011;342:D2111.
158. Wegmann TG, Lin H, Guilbert L, et al. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today. 1993;14(7):353–6.
159. Shaikh Z, Abbassi RM, Rizwan N, et al. Morbidity and mortality due to unsafe abortion in Pakistan. Int J Gynaecol Obstet. 2010;110(1):47–9.

160. Atrash HK, Hogue CJ. The effect of pregnancy termination on future reproduction. Baillieres Clin Obstet Gynaecol. 1990;4(2):391–405.
161. Frank PI, Mcnamee R, Hannaford PC, et al. The effect of induced abortion on subsequent pregnancy outcome. Br J Obstet Gynaecol. 1991;98(10):1015–24.
162. Horvath S, Schreiber CA. Unintended pregnancy, induced abortion, and mental health. Curr Psychiatry Rep. 2017;19(11):77.
163. Crockett SA. Risks of mifepristone abortion in context. Contraception. 2006;74(2):174.
164. Gan C, Zou Y, Wu S, et al. The influence of medical abortion compared with surgical abortion on subsequent pregnancy outcome. Int J Gynaecol Obstet. 2008;101(3):231–8.
165. Charonis G, Larsson PG. Use of Ph/Whiff test or quickvue advanced Ph and amines test for the diagnosis of bacterial vaginosis and prevention of postabortion pelvic inflammatory disease. Acta Obstet Gynecol Scand. 2006;85(7):837–43.
166. Larsson PG, Platz-Christensen JJ, Dalaker K, et al. Treatment With 2% clindamycin vaginal cream prior to first trimester surgical abortion to reduce signs of postoperative infection: a prospective, double-blinded, placebo-controlled, multicenter study. Acta Obstet Gynecol Scand. 2000;79(5):390–6.
167. Bjartling C, Osser S, Persson K. The association between mycoplasma genitalium and pelvic inflammatory disease after termination of pregnancy. BJOG. 2010;117(3):361–4.
168. Sukhikh GT, Kasabulatov NM, Van'ko LV, et al. Ratio between the number Of Th1 And Th2 lymphocytes in the peripheral blood and concentration of proinflammatory cytokines in lochia of women with postpartum endometritis. Bull Exp Biol Med. 2005;140(6):672–4.
169. Wang WJ, Zhang H, Chen ZQ, et al. Endometrial Tgf-Beta, Il-10, Il-17 and autophagy are dysregulated in women with recurrent implantation failure with chronic endometritis. Reprod Biol Endocrinol. 2019;17(1):2.
170. Yuan J, Li J, Huang SY, et al. Characterization of the subsets of human Nkt-like cells and the expression of Th1/Th2 cytokines in patients with unexplained recurrent spontaneous abortion. J Reprod Immunol. 2015;110:81–8.
171. Larsson PG, Platz-Christensen JJ, Thejls H, et al. Incidence of pelvic inflammatory disease after first-trimester legal abortion in women with bacterial vaginosis after treatment with metronidazole: a double-blind, randomized study. Am J Obstet Gynecol. 1992;166(1 Pt 1):100–3.
172. Cao Y, Sun H, Zhu H, et al. Allogeneic cell therapy using umbilical cord MSCs on collagen scaffolds for patients with recurrent uterine adhesion: a phase I clinical trial. Stem Cell Res Ther. 2018;9(1):192.
173. Salazar CA, Isaacson K, Morris S. A comprehensive review of Asherman's syndrome: causes, symptoms and treatment options. Curr Opin Obstet Gynecol. 2017;29(4):249–56.
174. Zhou Q, Wu X, Dai X, et al. The different dosages of estrogen affect endometrial fibrosis and receptivity, but not SDF-1/CXCR4 axis in the treatment of intrauterine adhesions. Gynecol Endocrinol. 2018;34(1):49–55.
175. Salma U, Xue M, Md Sayed AS, et al. Efficacy of intrauterine device in the treatment of intrauterine adhesions. Biomed Res Int. 2014;2014:589296.
176. Peng X, Li T, Zhao Y, et al. Safety and efficacy of amnion graft in preventing reformation of intrauterine adhesions. J Minim Invasive Gynecol. 2017;24(7):1204–10.
177. Wang J, Ju B, Pan C, et al. Application of bone marrow-derived mesenchymal stem cells in the treatment of intrauterine adhesions in rats. Cell Physiol Biochem. 2016;39(4):1553–60.
178. Reddy YS, Ramalaksmi BA, et al. Lead and trace element levels in placenta, maternal and cord blood: a cross-sectional pilot study. J Obstet Gynaecol Res. 2014;40(12):2184–90.
179. Frias-Espericueta MG, Cardenas-Nava NG, Marquez-Farias JF, et al. Cadmium, copper, lead and zinc concentrations in female and embryonic pacific sharpnose shark (Rhizoprionodon Longurio) tissues. Bull Environ Contam Toxicol. 2014;93(5):532–5.
180. Alebic-Juretic A, Frkovic A. Plasma copper concentrations in pathological pregnancies. J Trace Elem Med Biol. 2005;19(2-3):191–4.

181. Shen PJ, Gong B, Xu FY, et al. Four trace elements in pregnant women and their relationships with adverse pregnancy outcomes. Eur Rev Med Pharmacol Sci. 2015;19(24):4690–7.
182. Popovic JK, Grujic Z, Grujic I, et al. Prostaglandin E2, trace elements and levels of oxidative processes in spontaneous miscarriages. Eur Rev Med Pharmacol Sci. 2016;20(22):4786–90.
183. Prema K. Predictive value of serum copper and zinc in normal and abnormal pregnancy. Indian J Med Res. 1980;71:554–60.
184. Turan K, Arslan A, Uckan K, et al. Change of the levels of trace elements and heavy metals in threatened abortion. J Chin Med Assoc. 2019;82(7):554–7.
185. Ergaz Z, Guillemin C, Neeman-Azulay M, et al. Placental oxidative stress and decreased global DNA methylation are corrected by copper in the cohen diabetic rat. Toxicol Appl Pharmacol. 2014;276(3):220–30.
186. Vukelic J, Kapamadzija A, Petrovic D, et al. Variations of serum copper values in pregnancy. Srp Arh Celok Lek. 2012;140(1-2):42–6.
187. Zheng G, Zhong H, Guo Z, et al. Levels of heavy metals and trace elements in umbilical cord blood and the risk of adverse pregnancy outcomes: a population-based study. Biol Trace Elem Res. 2014;160(3):437–44.
188. Al-Jameil N, Tabassum H, Al-Mayouf H, et al. Analysis of serum trace elements-copper, manganese and zinc in preeclamptic pregnant women by inductively coupled plasma optical emission spectrometry: a prospective case controlled study in Riyadh, Saudi Arabia. Int J Clin Exp Pathol. 2014;7(5):1900–10.
189. Dickinson N, Rankin J, Pollard M, et al. Evaluating environmental and social influences on iron and zinc status of pregnant subsistence farmers in two geographically contrasting regions of Southern Malawi. Sci Total Environ. 2014;500-501:199–210.
190. Jameson S. Zinc and copper in pregnancy, correlations to fetal and maternal complications. Acta Medica Scand Suppl. 1976;593:5–20.
191. Swanson CA, King JC. Zinc and pregnancy outcome. Am J Clin Nutr. 1987;46(5):763–71.
192. Omeljaniuk WJ, Socha K, Borawska MH, et al. Antioxidant status in women who have had a miscarriage. Adv Med Sci. 2015;60(2):329–34.
193. Malavolta M, Piacenza F, Basso A, et al. Serum copper to zinc ratio: relationship with aging and health status. Mech Ageing Dev. 2015;151:93–100.
194. Thaker R, Oza H, Shaikh I, et al. Correlation of copper and zinc in spontaneous abortion. Int J Fertil Steril. 2019;13(2):97–101.
195. Kort KC, Schiller HJ, Numann PJ. Hyperparathyroidism and Pregnancy. Am J Surg. 1999;177 (1):66–8.
196. Amaya Garcia M, Acosta Feria M, Soto Moreno A, et al. Primary hyperparathyroidism in pregnancy. Gynecol Endocrinol. 2004;19(2):111–4.
197. Harsoulis F, Karayiannis B, Karvounaris D, et al. Primary hyperparathyroidism in pregnancy. J Obstet Gynaecol. 2000;20(2):188–9.
198. Gidiri M, Lindow SW, Masso EA, et al. Parathyroidectomy in pregnancy for primary hyperparathyroidism with successful pregnancy outcome: a report of two pregnancies. J Obstet Gynaecol. 2004;24(3):318–9.
199. Tollin SR. Course and outcome of pregnancy in a patient with mild, asymptomatic, primary hyperparathyroidism diagnosed before conception. Am J Med Sci. 2000;320(2):144–7.
200. Norman J, Politz D, Politz L. Hyperparathyroidism during pregnancy and the effect of rising calcium on pregnancy loss: a call for earlier intervention. Clin Endocrinol. 2009;71(1):104–9.
201. Borella P, Szilagyi A, Than G, et al. Maternal plasma concentrations of magnesium, calcium, zinc and copper in normal and pathological pregnancies. Sci Total Environ. 1990;99 (1-2):67–76.
202. Sami AS, Suat E, Alkis I, et al. The role of trace element, mineral, vitamin and total antioxidant status in women with habitual abortion. J Matern Fetal Neonatal Med. 2019;2019:1–8.
203. Sun Q, Zhang XL. Research on apoptotic signaling pathways of recurrent spontaneous abortion caused by dysfunction of trophoblast infiltration. Eur Rev Med Pharmacol Sci. 2017;21(3 Suppl):12–9.

204. Kapil U, Pandey RM, Jain V, et al. Status of iodine deficiency disorder in district Udham Singh Nagar, Uttarakhand State India. Indian J Endocrinol Metabol. 2014;18(3):419–21.
205. Narasinga Rao BS. Anaemia and micronutrient deficiency. Natl Med J India. 2003;16(Suppl 2):46–50.
206. Pathak P, Kapil U, Kapoor SK, et al. Prevalence of multiple micronutrient deficiencies amongst pregnant women in a rural area of Haryana. Indian J Pediatr. 2004;71(11):1007–14.
207. Nwaru BI, Hayes H, Gambling L, et al. An exploratory study of the associations between maternal iron status in pregnancy and childhood wheeze and atopy. Br J Nutr. 2014;112 (12):2018–27.
208. Serdar Z, Gur E, Develioglu O. Serum iron and copper status and oxidative stress in severe and mild preeclampsia. Cell Biochem Funct. 2006;24(3):209–15.
209. Hosnedlova B, Kepinska M, Skalickova S, et al. A summary of new findings on the biological effects of selenium in selected animal species-a critical review. Int J Mol Sci. 2017;18:10.
210. Kocak I, Aksoy E, Ustun C. Recurrent spontaneous abortion and selenium deficiency. Int J Gynaecol Obstet. 1999;65(1):79–80.
211. Thomas VV, Knight R, Haswell SJ, et al. Maternal hair selenium levels as a possible long-term nutritional indicator of recurrent pregnancy loss. BMC Womens Health. 2013;13:40.
212. Kumar KS, Kumar A, Prakash S, et al. Role of red cell selenium in recurrent pregnancy loss. J Obstet Gynaecol. 2002;22(2):181–3.

Chapter 12
The Variations of Metabolic Detoxification Enzymes Lead to Recurrent Miscarriage and Their Diagnosis Strategy

Chunlan Song and Wei Shang

Abstract Spontaneous abortion has been a common obstetrical and gynecological disease, which occurs in 10–15% of all pregnancies. Recurrent miscarriage (RM) refers to the occurrence of three or more times abortions with the same partner. It is generally believed that environmental pollution associated with economic development may cause infertility and RM. When xenobiotics from the environment enter the body, they must be cleared from the body by various metabolic enzymes in the body. The absence or variation of these enzymes may be the genetic basis of RM caused by environmental pollution. The variation of metabolic detoxification enzyme can directly affect the removal of harmful substances from internal and external sources. Therefore, the determination of metabolic enzyme activity may become an important factor in the diagnosis of RM etiology and seeking methods to improve the detoxification ability has a great significance for the treatment of RM.

Keywords Recurrent miscarriage · Environmental pollution · Metabolic detoxification enzymes

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12.1 Overview of Recurrent Spontaneous Abortion

With the increasing attention and increasing deep understanding of recurrent spontaneous abortion, some clinical guidelines and practitioners expand the definition of recurrent spontaneous abortion to two or more times continuous spontaneous miscarriages [1], which makes the population with this disease larger. The biggest change lies in improving the understanding of the complex and multifactorial morbidity of recurrent spontaneous abortion. In recent years, etiological studies of recurrent spontaneous abortion have covered a variety of pathogenic factors. In addition to previous opinion that recurrent spontaneous abortion is caused by genetic material abnormality, anatomic structure abnormality of genital tract, endocrine system abnormality, autoimmunity abnormality, infection factors and thrombotic diseases [2], the adverse effects of environmental pollution and metabolic and detoxifying enzyme on female fertility have also been taken seriously in recent years [3], thus further expand the scope of research and management of recurrent spontaneous abortion. Although it has not been taken into account as a cause of disease in the guidelines of most international academic organizations, environmental factor, as one of the potential predisposing factors for recurrent spontaneous abortion of couples getting ready for pregnancy, cannot be ignored, which will be highlighted later in this chapter.

In addition to the above-mentioned traditional causes of disease, in recent years, more and more evidences show that many chemicals and other environmental pollutants, including air, natural gas, and daily touched articles, directly or indirectly interact with traditional pathogenic factors to interfere with epigenetics by affecting human endocrine system and even DNA methylation status of the offspring, thus constituting a major threat to human reproduction health. Exposure to environmental pollutants in fetal period, neonatal period, adolescence or adulthood can cause impact on reproductive health and other aspects, which undoubtedly becomes a potential predisposing factor for recurrent spontaneous abortion and may have intergenerational impact. Most chemicals have not been evaluated for hazard rating like drugs, so they are rarely regarded as harmful to human health. Therefore, clinicians need to improve the clinical ability of prevention and treatment of repeated spontaneous abortion caused by environmental factors.

12.2 Mechanism of Recurrent Spontaneous Abortion Caused by Environmental Pollution

According to current studies, it is believed that recurrent spontaneous abortion is mainly caused by environmental factors, genetic factors, and interaction of the two. Many exposure factors in the environment, such as environmental endocrine disruptors, maternal malnutrition during pregnancy, maternal diseases, and maternal lifestyle (smoking, drinking, etc.), can act on the developing embryo or fetus and lead to repeated spontaneous abortion by changing the dynamic balance of maternal

hormones or directly affecting its normal development. Genetic material abnormality mainly includes gene mutation and chromosome aneuploidy. The parental generation can transfer abnormal genetic material to the offspring, which may lead to embryo abnormality and loss. This chapter briefly describes the main environmental factors and genetic factors that may lead to recurrent spontaneous abortion and focuses on exploring the potential pathogenesis of recurrent spontaneous abortion from the aspect of the interaction between environmental factors and genetic factors.

12.2.1 Basic Concepts

1. Environmental Endocrine Disruptor

Environmental Endocrine Disruptors (EEDs), also known as environmental hormones, are chemicals that exist in the environment and have the ability to interfere with the normal signal transduction system in human body. They can imitate, block or regulate the synthesis, release, transportation and metabolism process of natural hormones, thus affecting the genital system, nervous system, endocrinium and immunity system of organism or human body.

EEDs include persistent pollutants, pesticides, a wide range of industrial compounds (such as phthalates and bisphenol A) and their decomposition products. It should be noted that not all EEDs are man-made compounds. Many plant-produced substances (plant hormones) can also have different endocrine effects, whether harmful or beneficial.

Although EDDs in the environment may be only at very low level, they may still endanger the whole body and the offspring, even the health of the whole population or subgroup; especially when several different compounds act on the same goal, it is more harmful. It is more important to state briefly that embryos and fetuses at the growth and development stage are more vulnerable to the damage of EDDs.

At present, there are more than 60 kinds of chemical substances listed as EDDs in the world. They are widely distributed, have many action links, and even have biological concentration, bioaccumulation, and biomagnification in metabolism and it is very difficult to prevent its harm, so it is very important to improve the awareness of its harms and improve the public awareness of environmental protection.

2. Gene-Environment Interaction

Human health and diseases are the result of the joint action of heredity (gene) and environment. Genetic and/or environmental threats corresponding to certain transient and persistent health hazards are bound to be found. Gene-Environment Interaction (GXE) means that different genotypes have different effects on the environment. When environment and genetic factors coexist, the combined effect of the two is not equal to the sum of effects of single factors, so it is considered that there is interaction between the two kinds of factors. The combined action of genetic and environmental factors is not only reflected as risk factors jointly responsible for

the occurrence of existing related diseases, but also reflected in the epigenetic changes of heredity due to environmental impact; and it also passes this change on to future generations.

12.2.2 Mechanism of Action

EEDs, as a kind of exogenous bioactive substances, must go through actions of a variety of internal metabolic enzymes before they are finally removed from human body. If human body cannot timely and effectively discharge the exogenous harmful substances, after a long time of accumulation, the genetic material involved cannot exist stably, which will lead to cancerization of somatic cells, embryonic dysplasia and even loss.

Different studies show that the impact of environmental factors on female fertility varies greatly and their impact on female reproductive system is very wide. Women diseases that may be caused by exposure to exogenous bioactive substances include: (1) Ovarian dysfunction: chromosomal aneuploidy, polycystic ovarian syndrome, endometriosis and menstrual cycle changes; (2) Uterine diseases: leiomyoma of uterine; (3) placenta dysfunction and adverse pregnancy outcome: early pregnancy loss, recurrent spontaneous abortion, fetal growth restriction; (4) breast diseases: breast cancer, shortened lactation period, and premature thelarche in puberty. This chapter focuses on the relevant mechanism of recurrent spontaneous abortion caused by EEDs.

12.2.2.1 Recurrent Spontaneous Abortion Caused by Genetic Factors

The genetic materials determine the developmental characters of organism. The parental generation can pass abnormal genetic materials to the offspring, which may lead to abnormal characters of the offspring. Genetic material abnormality mainly includes gene mutation and chromosome aberration.

1. Gene Mutation

Gene mutation is the change of gene structure caused by insertion, deletion or replacement of base pairs in DNA molecule. It can occur at any stage of development but has a low correlation with recurrent spontaneous abortion. It has been found in studies that gene mutation is related to DNA replication, DNA damage repair, cancerization, and so on, and may lead to polycystic kidney, limb deformity, and so on [4].

2. Chromosome Aberration

Chromosomal aberration refers to the increase or decrease of chromosome number or the change of structure, that is, numerical aberration and structural aberration.

Aneuploidy, namely, numerical abnormalities of chromosomes, is the main cause of recurrent spontaneous abortion. In humans, meiosis begins in the ovaries in fetal period, but stagnates in the diplonema of the first meiosis, and does not resume until ovulation. Chromosomal aneuploidy causes embryo or fetus loss by interfering with the meiosis process. The survival rate of fetuses with reduced chromosome number, i.e. monosomic fetuses, is very low. Most of them die in the embryonic period and the survivals are often accompanied with malformations, such as congenital agenesis of ovaries (Turner's Syndrome) [5]; the increased chromosome number can also lead to malformations, most of which are trisomy. For example, Trisomy 13, 18, 21 Syndrome is mostly manifested as growth retardation, mental retardation, slow response, and so on [5].

Chromosomal structural aberration refers to the phenomenon that chromosome breaks and reconnects in abnormal combinations and can be divided into deletion, repetition, inversion, translocation, and so on. This type of aberration is more common in children with genetic defects, such as Fragile X Syndrome [6] caused by fracture of fragile part of X chromosome, Williams Syndrome caused by deletion of elastin gene region of Chromosome 7 code, and cri-du-chat syndrome caused by the fracture and deletion of the end of the short arm of Chromosome 5, and its manifestations are often growth retardation, mobility inconvenience, mental retardation, and so on [6].

12.2.2.2 Recurrent Spontaneous Abortion Caused by Gene-Environment Interaction

1. Typical Endocrine Disruptor

The traditional view is that recurrent spontaneous abortion is mainly related to genetic defects; however, recent studies show that environmental threats play a crucial part in the occurrence of recurrent spontaneous abortion, such a bad pregnancy outcome, especially EEDs are most studied in last several years and also a kind of environmental exposure factors that have a great influence on the normal development of fetus in maternity. Lots of studies have proved that EEDs can cause premature birth, low birth weight, obesity, metabolic disorders, and urogenital abnormalities of offspring [7]. The accumulated EEDs with great harm to human body are Organochlorine Pesticides (OCPs), Phthalic Acid Esters (PAEs), and Bisphenol A (BPA).

a. OCPs

Some studies [7] have pointed out that chemicals for agricultural purpose, especially OCPs, are one of the important factors of birth defects and recurrent spontaneous abortion. After entering human body, OCPs can interfere with the normal functions of thyroxin, estrogen, androgen, insulin, and neuroendocrine system, so as to indirectly affect the normal operation of the human body's reproductive system, cardiovascular system, and metabolic system, thus resulting in birth defects or embryo loss. Some studies have confirmed that in terms of parturients who

had been exposed to DDT and HCB, the serum follicle-stimulating hormone (FSH), estradiol (E_2), and progesterone (P) in maternal blood and FSH, luteinizing hormone (LH) and E_2 in cord blood are positively related to the levels of residual DDT and HCH in human body, that is, there is a clear dose-response relationship; however, LH in maternal blood and P in cord blood are negatively related to the levels of residual DDT and HCH in human body, that is, there is also a dose-response relationship, which indicates DDT and HCH in human body may interfere with the normal sex hormone level. In addition, the mRNA expression levels relevant genes in placenta tissue (α-ER, β-EP, GnRH) and umbilical cord tissue (α-ER, β-EP) are also positively related to the levels of residual DDT and HCH in human body, that is, there is a dose-response relationship. The results show that OCPs can destroy the normal hormone level and interfere with the expression of relevant genes, which further leads to reproductive disorders, embryo loss or birth defects of offspring [8]. Some studies have found that exposure to methoxychlor (MXC) can reduce the number of successful implantation in uterus of pregnant rats; after implantation, MXC has embryotoxicity to embryos, thus resulting in increased adverse pregnancy outcomes such as stillborn and absorbed embryos.

b. BPA

Some teams found that BPA penetrate the placenta barrier relying on its lipid solubility to do harm to embryonic cells and cause abnormal differentiation and loss of embryos. At the same time, in vitro experiments also found that with the increase of BPA concentration, in vitro growth and development of embryos were more and more seriously affected, showing a dose-response relationship; the increase of BPA concentration could also induce poor growth of yolk sac and vascular differentiation, slow growth and morphological differentiation abnormality, increasing the possibility of embryo loss.

c. PAEs

A large number of clinical tests and animal experiments have confirmed that PAEs can penetrate the placental barrier and produce embryotoxicity to fetus in uterus after entering human body. Shiota et al. [9] researched the harmful effects of average daily and high exposure dose to PAEs on pregnant rats and the results showed that high-dose exposure to PAE substances, such as Dibutyl Phthalate (DBP) and Bis (2-ethylhexyl) phthalate (DEHP), could affect the normal development of fetal rats in pregnancy and there was a significant dose-effect relationship between the mortality rate and birth weight of fetal rate born and the exposure dose of PAEs, indicating that high-dose exposure to PAEs would lead to an increase in spontaneous abortion rate of fetal rats. In an investigation to newborns Latini et al. [3] found that the probability of DEHP or MEHP in cord blood of newborns was as high as 88.1%, indicating the universality of daily exposure to PAEs and its embryotoxicity; moreover, in terms of gestational period, mothers of newborns with positive MEHP test results had shorter gestational period than those with negative MEHP test result, which means PAEs may lead to premature delivery. Through animal experiments, Saillenfait et al. [3] found that Diisooctyl Phthalate (DIOP) with anti-androgen effect could affect sex differentiation of fetal rats in

uterus of pregnant rats by interfering with androgen-dependent reproductive development, which results in the increase of the number of absorbed fetuses and causes serious distortion of the reproductive system of fetal rats, thus leading to embryos loss.

2. Basic function channels of environmental factors

Exposure factors in the environment can play their roles in at least three ways [10]: (1) Direct action: EEDs can directly bind to hormone receptors and have an impact on the reproductive system after entering human body or they can firstly bind to other receptors in human body, jointly act on hormone receptors, and then have an impact on the reproductive system. (2) Neuroendocrine approach: the nervous system feeds back regulatory signals to the endocrine system by monitoring EEDs. (3) Epigenetic approach: they change the transcription activity of DNA but do not change its sequence.

3. EEDs and epigenetics

The harm of EEDs to health is not only limited to the early developmental phase or a certain stage of life process. EEDs can lead to the reprogramming of the epigenetics of somatic cells and germ cells and continuous changes of gene expression, thus promoting intergenerational heredity.

DNA methylation, histone modification, and microRNA are the main epigenetic ways. Although they do not change DNA sequence, they can change DNA function and pass it on to the next generation or even the generation after the next. It has been confirmed by animal experiments that reprogramming of epigenetics causes spontaneous abortion in the offspring of exposed maternal rats; the changes in epigenetics affect the development of neuroendocrine system and produce abnormal behaviors; EEDs pass the harmful effect on between generations through epigenetics, which constitutes a long-term harm to health.

To sum up, recurrent spontaneous abortion can be roughly divided into three types according to its pathogenesis: recurrent spontaneous abortion due to genetic factors, recurrent spontaneous abortion due to environmental factors, and recurrent spontaneous abortion due to gene–environment interaction, and recurrent spontaneous abortion is mainly caused by gene–environment interaction. In recent years, with the aggravation of environmental pollution, the morbidity of recurrent spontaneous abortion has also been increasing. So more and more people pay attention to how to prevent recurrent spontaneous abortion. At present, relevant studies have shifted from single studies of environmental risk factors or genetic factors to the direction of gene–environment interaction to thoroughly understand the pathogenesis of recurrent spontaneous abortion and formulate more scientific and effective prevention means and measures, thus reducing the incidence of recurrent spontaneous abortion.

12.3 Roles of UGT Metabolic Enzymes in RM

12.3.1 A Brief Introduction to Detoxifying Enzymes in Human Body

Xenobiotics in the environment (mainly organic chemicals and parahormone pollutants) must be removed with a variety of metabolic enzyme after entering human body. If human body cannot quickly discharge the exogenous hazardous substances, genetic material instability [11], even teratogenetic embryonic development and somatic cell cancerization [12] will be caused under long-term effects.

There are mainly detoxification enzymes of three phases in human body, namely, I-Phase Cytochrome Oxidase P450 (CYPs) and Alcohol Dehydrogenase; II-Phase Detoxification Enzymes include Glutathione Reductases (GSTs), Acetylase (NAT1 and NAT2), UDP-glucuronyltransferases (UGTs), Methyltransferase, Aminotransferases, and Sulfate Transferase (SULTs); III-Phase Detoxification Enzymes are transferases. Previous studies have shown that relevant gene deletions of CYPs and GST are correlated with RM [13–15].

12.3.2 Introduction to UGTs

12.3.2.1 What are UGTs

UDP-glycosyltransferases (UGTs) are superfamily enzymes, which exist in animals, plants, fungi, and bacteria [16]. UGT is one of the most important enzymes for II-Phase biotransformation and belongs to the Glycosyl Transferase Superfamily. In mammals, UGT Superfamily includes four families: UGT1, UGT2, UGT3, and UGT8 (Fig. 12.1). UGTs include many subtypes which are the results of gene duplication and differentiation. So far, 22 subtypes have been found in human beings [16].

Enzymes in UDP-Glycosyltransferase (UGT) Superfamily catalyze glucuronidation of various compounds, with multiple substrates, including endogenous compounds (e.g., bilirubin, steroid hormones, etc.) and exogenous compounds (e.g., phenols, non-steroidal anti-inflammatory drugs, etc.). UGTs take UDP-glucuronic acid as the glycosyl donor to react with various functional groups (hydroxyl, carboxyl or amino are most common) in the substrate (often nonpolar), so as to increase their water solubility, thus promoting their excretion from human body through feces or urine [17].

UGTs are widely distributed in various tissues of human body, such as liver, kidney, intestine, brain, skin, and so on. The activity of UGTs is the highest in liver. The main function of UGTs is biotransformation. Such biotransformation plays a key role in eliminating numerous potential toxic exogenous chemicals, removing

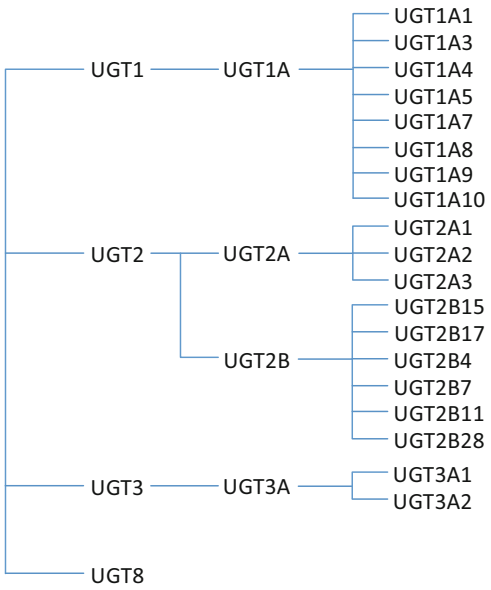

Fig. 12.1 Human UDP-glycosyltransferases (UGTs)

toxic endogenous metabolites, and controlling the distribution and level of endogenous signal molecules [16].

UGT1 and UGT2 play an important part in pharmacology and toxicology. Their loss or change in activity may lead to disposition differences of drugs among individuals and increased cancer risk [18]. These UGTs are highly expressed in detoxification apparatus such as liver, kidney, and intestine, and their expression level varies with the detoxification needs of human body. The difference between UGT3 and UGT8 enzymes and UGT1 and UGT2 enzymes is that the glycosyl donors are different. UGT1 and UGT2 enzymes mainly use UDP-glucuronic acid; while UGt3 enzymes use UDP-Glucose, UDP-Xylose, and UDP-N-acetylglucosamine; and UGT8 enzymes only use UDP-Galactose. Their functions and regulatory mechanisms still need further study [16].

12.3.2.2 Effect of UGTs:

1. Role of UGTs in drug reactions

UGTs participate in the drug metabolism process and help human body to clear a great number of usually used drugs. Their genovariation leads to individual differences in drug clearance level, which has important clinical significance in pharmacology. In addition, many drugs can enhance or inhibit the activity of UGTs, thus changing the ability to metabolize other drugs, namely drug–drug interaction [16]. UGT gene polymorphism causes changes in drug clearance rate during

pregnancy [19], which in turn affects embryonic development. The drug clearance rate during pregnancy is correlated with the number of pregnancies [20].

2. Role of UGTs in metabolizing environmental toxins

After entering human body, many toxic substances in the environment, such as bisphenol A and polychlorinated biphenyl, must be excreted from human body after increasing hydrophilicity by metabolism of UGTs [21]. The abnormality or deletion of UGTs in human body is closely related to the efficacy, toxicity, some diseases, as well as treatment. When the activity of UGTs is inhibited, endogenous and exogenous substances metabolized by UGTs may accumulate in human body, which has an impact on human body [22]. Glucuronidation of UGTs can protect human body from damage of environmental toxins, especially damage to embryonic development.

3. Role of UGTs in metabolizing endogenous compound including signal molecules

UGTs not only play an important part in pharmacology and toxicology, but also play an important role in the metabolism of endogenous small molecules, so as to maintain the homeostasis of human body. These molecules include normal metabolites, hormones, signal molecules, and some lipids [16]. In the process of bilirubin metabolism, the hereditary variation of UGT1A1 gene expression results in the decrease or nonexistence of UGT1A1 level in liver, thus resulting in the increase of unconjugated bilirubin, namely, hyperbilirubinemia, and even rare Crigler Najjar (CN) syndrome [23, 24]. In the process of sex hormone metabolism, UGTs exist in many target sites where sex hormones are composed and play their roles. Glucuronidation of UGTs can make sex hormones inactivated and excreted from human body, so that human body can maintain normal hormone level, which is of great significance to hormone-dependent cancers [25].

4. Role of UGTs in cancer protection and cancer risk

Studies have confirmed that due to UGTs' features of combining with environmental toxins, carcinogens, and hormones, changes in variation or activity of UGTs may increase the risk of suffering from cancers or promote their progress [26–28]. Numerous case-control studies have shown that UGT gene polymorphism is a risk factor for many cancers.

12.3.2.3 Regulation of UGT Levels

In terms of tissue-specific expression of human UGTs, the levels of UGT RNA or protein detected in human tissues vary greatly, especially in the liver. Controlling the expression of UGTs plays an important role in drug response, detoxification and maintaining homeostasis. UGTs are regulated by both endogenous and exogenous signals. Metabolic activity of UGTs can be changed by some drugs and poisons and the expression of UGTs can be increased or decreased by steroid hormones, bile acids or exogenous biological agents through sensors (transcription factors on which

the receptor depends). This enables UGTs to respond to the demands of cells and tissues for detoxification and maintaining the steady state of endogenous signaling molecule level. In addition, the functions of UGTs can be further diversified through alternative splicing and oligomerization [16].

Gene expression and catalytic activity of UGTs are related to gene copy number and single nucleoside deletion. UGT2B15 and UGT2B17 gene deletions are related to sex hormone metabolism [29]. In the studies of UGT2B17 chimeras, it is found that the amino acid sequence 61-194 of UGT2B15 is responsible for specific binding with the substrate [30], suggesting that the deletion of UGT2B15 may have different effects on metabolism. Stringer et al. [31] studied the pharmacokinetics of sipoglitazar drug in human body, according to which UGT2B15 gene deletion has a significant impact on its metabolism.

12.3.3 Common Environmental Pollutants, UGTs, and Embryonic Development

1. Bisphenol A (BPA)

BPA is a basic raw chemical material widely used in the production of various plastics. BPA is a liposoluble organic compound. If beverages and food are packed in plastic containers, high fat content or heating may promote the release of BPA into food. After being absorbed into the blood through the skin or gastrointestinal tract, BPA, as an endocrine disruptor, is mainly oxidized in the liver and combined with glucuronic acid, detoxified under the action of UDP-glucuronyltransferase (UGT) and excreted from human body with urine. Glucuronidation of BPA is mainly completed by UGT2B15 [32].

BPA can affect the reproductive system and result in chromosomal abnormalities in eggs [33]. BPA has estrogen-like effects and even BPA at low concentration may interfere with normal embryonic development and may cause birth defects and long-term impact of fetuses [34]. BPA can competitively inhibit UGT2B4, noncompetitively inhibit UGT2B7, 2B15 and 2B17. Therefore, BPA may not only play its estrogen-like activity, but also give play to a double toxic effect by leading to metabolic abnormalities due to inhibition UGT subtypes [35]. Nishikawa et al. [36] found that low-dose BPA can affect embryonic development in the experimental study of rats. BPA entering the intestine of rats was absorbed into their liver, and then transformed into non-toxic BPA-G by UDP-glycosyltransferase (UGT). After it enters the embryo through the placenta with the transporter, organs with high GUSB activity, such as embryo lung and intestine, may dissociate the glucuronic acid of BPA-G and generate BPA which may give play to its toxic effect on the target organs of the embryo.

2. Polychlorinated Biphenyls (PCBs)

Polychlorinated Biphenyls are a kind of chlorinated organic compounds formed by replacing hydrogen atoms in biphenyl molecules with some chlorine atoms. They are stable in nature and are not easy to hydrolyze and oxidize, so that they are widely used in industrial production. PCBs mainly enter human body with industrial wastewater and municipal wastewater and have biological concentration effect.

PCBs are typical EEDs with estrogen-like effects. Maternal contact with PCBs can lead to abnormality of offspring in development and postnatal behaviors. It is found in studies that PCBs can reduce thyroxin level during the developmental phase of human body, cause slow weight growth, hearing loss, and damage to immunologic function, growth and development disorders, and increased incidence of some cancers. In the famous "Rice Bran Oil Poisoning Incident" occurred in 1968 in Japan, after pregnant women eat rice bran oil contaminated by PCBs, situations such as fetal death and abnormal development of fetuses and occurred.

Polychlorinated Biphenyls (PCBs) are good substrates for UGTs. The toxicity of PCBs can be reduced or relieved through glucuronidation of UGTs and there is a structure-function relationship between them [37, 38]. UGT1A1, 1A6, and 2B1 are the main UGT subtypes that can catalyze glucuronidation of PCBs. In addition, PCBs are also inhibitors of UGTs and they can affect metabolism elimination by inhibiting activity of UGTs [21]. When the level of PCBs in human body is too high or the gene variation or expression of UGTs are abnormal, human body cannot metabolize the PCBs, which causes PCBs accumulate in human body and pass through the placental barrier, thus affecting fetal development, resulting in arrested fetal development, and so on.

3. Phthalic Acid Esters (PAEs)

PAEs can cause human contact through skin absorption, inhalation, drug injection, and oral administration [39]. PAEs go through a two-step metabolic elimination process in the human body. PAEs are initially converted from lipase to monoester metabolites by phase I metabolism. These monoesters are then catalyzed by UDP-glycosyltransferase (UGT) and react with UDP-glucuronic acid (UDPGA) to form glucuronic acid conjugates [40]. For simple PAEs with short branched chain, about 70% of excreted monoesters are unconjugated and similar glucuronidation patterns have been found in plasma. PAEs studies in vitro and vivo show that PAEs have stronger biological activity and toxicity [41], so studies of the toxicity mechanism of Phthalic Acid Esters is of great significance.

As a kind of EEDs, PAEs have serious impact on human hormone regulatory system, such as thyroid hormone, steroid hormone, androgen, etc. PAEs were reported to be significantly negatively correlated with thyroid stimulating hormone (TSH), triiodothyroxine (T3), thyroxine (T4), and free thyroxine (FT4), but the specific mechanism is still not clear [42]. In addition, PAEs in human milk can reduce androgen activity and interstitial cell functions [42]. Phthalic Acid Esters may also cause premature breast development because of their estrogen and antiandrogen activities. PAEs reduce estradiol level through reducing the gene transcription level of aromatase [43].

PAEs can inhibit the activity of UGT1A6, UGT1A7, and UGT2B4 to a certain extent, while UGT1A9 is widely inhibited by PAEs, which indicates that there may be a good interaction between Phthalic Acid Esters and UGTs [44]. In the study on the inhibition of human UGTs activity by phthalic acid monomer, it was proved that PAEs had high specific inhibition on UGT1A7 and UGT1A9 [45]. PAEs may interfere with signal transduction and drug metabolism of endogenous hormones through inhibiting UGTs, while the embryonic development requires the maintenance of normal hormone level in human body. The hormone abnormality caused by PAEs is bound to affect the embryonic development, so close monitoring of PAE exposure should be paid attention to, so as to reduce the risk of disease caused by PAEs.

12.4 Treatment of Recurrent Spontaneous Abortion

For patients with RSA, clinicians should inquire about the medical history in detail and conduct etiological screening with assistance of necessary laboratory detections. RSA treatment is mainly carried out according to causes of the disease and experimental treatment can be carried out for a few patients with URSA. After pregnancy, monitoring and management should be strengthened for patients with RSA, and pregnancy should be terminated timely.

12.4.1 Anatomic Structure Abnormality

1. Cervical incompetence

Cervical cerclage is the main treatment for cervical incompetence and can effectively prevent premature delivery before 34 weeks of pregnancy. It has been reported that after a meta-analysis of the clinical data of 2091 patients, it is found that cervical cerclage may reduce the pregnancy loss rate and neonatal mortality rate of single-birth pregnant women at the risk of premature delivery. It is pointed in the Guidelines to Diagnosis and Treatment of Cervical cerclage issued by ACOG: Cervical cerclage can be performed to single-birth pregnant women who underwent more than once painless cervical dilatation, have no medical history of abortion in the second trimester during labor or no placental abruption, or underwent cervical cerclage in the previous pregnancy due to painless cervical dilatation in the 13th to 14th week of pregnancy, that is, preventive cervical cerclage [46].

2. Congenital dysgenesis of uterus

At present, there are no relevant controlled trial studies of surgical treatment of uterine malformation to improve pregnancy outcome. At the same time, it is believed in RCOG's Guidelines that there is no sufficient evidence to support that

transcervical resection of septum can effectively prevent RSA patients from spontaneous abortion again.

3. Other uterine diseases

Due to the changes in morphology of uterine cavity, diseases such as intrauterine adhesion and submucous myoma are not conducive to the implantation, growth, and development of fertilized eggs, which may also be the causes of RSA. Therefore, some scholars suggest that transcervical adhesion separation surgery should be performed to RSA patients with adhesions. After the surgery, IUD should be placed to prevent re-adhesions or estrogen and artificial cycles should be used periodically to promote the growth of endometrium. Transcervical resection of myoma should be performed to patients with submucous myoma before pregnancy. In terms of patients with large intramural myoma, myomectomy should be performed. However, the "ESHRE Guidelines" suggest that it is unproven that transcervical resection of polyp or submucous myoma have more benefits for RSA. New studies show that, for those with high risk of infertility or spontaneous abortion caused by endometrial damage, the endometrial growth can be promoted by transplanting umbilical cord mesenchymal stem cells cultured with collagen scaffolds into the uterine cavity [47].

12.4.2 Prethrombotic State

The "ESHRE Guidelines" recommend using Low Molecular Weight Heparin (LMWH) alone or in combination with low-dose aspirin (LDA) in PTS treatment. The general usage of LMWH is 5000 U subcutaneous injection once or twice a day. The medication time can start from early pregnancy and generally start from the diagnosis of pregnancy by detecting blood B-HCG. In the treatment process, if good fetal development is monitored, the medication can be stopped after abnormal indicators related to prethrombotic state return to normal. After the medication is stopped, the relevant indicators of prethrombotic state shall be reexamined regularly and the fetal growth and development shall be monitored. If there is any abnormality, restarting of medication shall be considered. If necessary, the treatment can be continued to the whole pregnancy period, and the medication shall be stopped 24 h before the termination of pregnancy.

For the patients with acquired hyperhomocysteinemia, a certain effect can be achieved through supplementation of folic acid and Vitamin B12. Glue et al. found that L-Methylfolate, Vitamin B6, and Vitamin B12 can reduce homocysteine level and even make 76% of patients reach normal level [48].

12.4.3 Chromosomal Abnormality

Balanced/unbalanced translocation of parental chromosomes can be detected through karyotype analysis of peripheral blood chromosomes, and karyotype abnormality of embryos can be detected through embryo biopsy or embryo product detection. The "ESHRE Guidelines" recommends conducting genetic counseling for couples with recurrent spontaneous abortion caused by chromosomal abnormalities, informing the recurrence risk, and suggesting solving the fertility problem through assisted reproductive technology. Preimplantation Genetic Diagnosis (PGD) is often used to detect the embryos of patients with RSA caused by parental chromosome abnormalities; Preimplantation Genetic Screening (PGS) is usually used for RSA patients with normal parental chromosomes. Studies have shown that about 76.5% of the couples choose Preimplantation Genetic Diagnosis (PGD), which greatly reduces the pregnancy loss rate [49].

12.4.4 Endocrine and Metabolism Abnormality

According to the American Society for Reproductive Medicine, patients with endocrine abnormalities, such as hyperthyroidism, Clinical Hypothyroidism (CH), Subclinical Hypothyroidism (SCH), and diabetes, should be actively monitored and treated before and during pregnancy.

1. Hyperthyroidism: It is suggested that RSA patients with a medical history of hyperthyroidism can be pregnant only after controlling their disease. Patients with mild hyperthyroidism should take antithyroid drugs, such as Propylthiouracil (PTU) which is relatively safe and will not increase the incidence of fetal malformation, during pregnancy.
2. Clinical Hypothyroidism: All RSA patients who have been diagnosed with hypothyroidism need to be treated with thyroid hormone. It is suggested that pregnancy should be considered after the thyroid function returns to normal for 3 months, and thyroid hormone should be taken during pregnancy.
3. Subclinical Hypothyroidism: Levothyroxine sodium should be supplemented as appropriate to control Thyrotropic Hormone (TSH) to normal level, and iodine can be supplemented properly.
4. Diabetes: It is recommended that the patients diagnosed with diabetes should take contraceptive measures before the blood glucose is not controlled, control the blood glucose within the normal range as far as possible 3 months before the planned pregnancy, and adopt insulin treatment instead of taking hypoglycemic drugs 3 months before the planned pregnancy.
5. PCOS: Whether PCOS causes RSA is still controversial. At present, it is unproven that metformin treatment can reduce the spontaneous abortion rate of RSA patients. Some studies show that metformin can reduce PCOS significantly or the pregnancy loss rate of women with insulin resistance and taking metformin

in early pregnancy does not increase the incidence of birth defects in fetuses [50]. At present, metformin is an empirical treatment for RSA patients with PCOS.

6. Corpus Luteum Insufficiency: Progesterone plays an indispensable role in the establishment and maintenance of pregnancy. Corpus Luteum Insufficiency may affect the maintenance of pregnancy. Supplementary treatment with micronized progesterone for vagina use 3 days after the LH peak can significantly improve the live birth rate of RSA patients with unknown causes. It is recommended that the patients with Corpus Luteum Insufficiency should complement corresponding hormones to normal level.

12.4.5 Infection

Genital tract infection is closely related to advanced RSA and premature delivery. Therefore, the patients with a medical history of genital tract infection should be routinely screened for bacterial vaginosis, mycoplasma, chlamydia, and so on before pregnancy. It is suggested that RSA patients with genital tract infection should be given specific treatment according to the type of pathogens before pregnancy, and can only be pregnant after the infection is controlled.

12.4.6 Immune Dysfunction

It is necessary to carry out targeted treatment according to the type of immune dysfunction of patients.

1. Autoimmune dysfunction

   a. Antiphospholipid syndrome (APS)

The diagnosis of typical APS must have at least one clinical standard, including: 3 times or above RSA less than 10 weeks of pregnancy; once or above spontaneous abortion after more than 10 weeks of pregnancy; once or above placental dysfunction diseases before 34 weeks of pregnancy; and at least one laboratory index, including: twice or above consecutive LA positive in the interval of 12 weeks or above; or ACA or anti-B2GP1 antibody titer >99th percentile.

At present, in terms of APS-related RSA patients, the first-line treatment is low-dose aspirin and low molecular weight heparin. A meta-analysis of the pregnancy outcomes of RSA patients with typical APS shows that after aspirin and heparin treatment, the fetal live birth rate of APS women was significantly increased and the spontaneous abortion rate was reduced to 54% [51]. In contrast, RSA patients with antiphospholipid antibody positive did not significantly reduce the risk of recurrent spontaneous abortion through using glucocorticoid and intravenous immunoglobulin. Therefore, RSA patients with primary APS should be given

anticoagulant therapy and it is not suggested to give hormone or immunosuppressor therapy.

Some experts have put forward the concept of atypical obstetric APS: (1) APL positive but atypical clinical manifestations (e.g., twice unexplained spontaneous abortions of less than 10 weeks of pregnancy; 3 time or above discontinuous unexplained spontaneous abortions); (2) patients with typical APS clinical manifestations but intermittently APL positive; (3) Laboratory APL indicators do not meet the middle and high titer positive (>99th percentile) but only low titer positive (95th to 99th percentile). Studies have shown that low-molecular-weight heparin treatment for patients with atypical obstetric APS may lead to good pregnancy outcomes [52]. Therefore, it is suggested that anticoagulant therapy can be carried out for patients with atypical obstetric APS. During the treatment, embryonic development should be closely monitored and APL should be reexamined regularly. When embryonic development is good and APL is negative for three consecutive times, drug withdrawal should be considered.

b. Antinuclear antibody positive

Patients with autoimmune diseases such as SLE should choose the right time to conceive after the remission of the disease under the guidance of physicians from both Rheumatology and Immunology Department and Obstetrics Department. During pregnancy, SLE activity and fetal development should be closely monitored; drugs should be used reasonably; and pregnancy should be terminated timely. It is suggested that RSA patients with antinuclear antibody positive should be treated with adrenal cortical hormone, prednisone 10–20 mg/day.

c. Antithyroid antibody positive

The increase of thyroid autoantibody titer may be related to the occurrence of pregnancy complications such as spontaneous abortion and premature delivery; however, there is few evidence of evidence-based medicine for intervention treatment. Therefore, at present, only regular monitoring of serum TSH level is available for pregnant women with thyroid autoantibody positive. When the TSH level increases and exceeds the reference range for pregnancy, thyroid will be given; however, for those with a medical history of RSA, we can take a more active treatment where appropriate. Low-dose-thyroxin therapy may be considered for RSA patients with thyroid autoantibody positive.

2. Alloimmune dysfunction

At present, there are many studies of the protective antibodies, that is, the lack of blocking antibodies and the increasing quantity and activity of NK cells. It is believed in previous studies that lymphocyte immunology thserapy (LIT) and intravenous immunoglobulin (IVIg) can significantly improve the pregnancy outcomes of patients with spontaneous abortion caused by alloimmune dysfunction or patients with unexplained recurrent spontaneous abortion (URSA). However, the long-term impact of Lymphocyte Immunology Therapy on mothers and infants remains to be followed up. A meta-analysis of five randomized controlled trials

(246 cases) shows that Intravenous Immunoglobulin cannot increase the live birth rate of RSA patients (OR = 0.98; 95% CI, 0.45–2.13). At present, there is a great controversy about the effectiveness of the two immunology therapies, LIT and Intravenous Immunoglobulin. According to the 2011 RCOG Guidelines, immunology therapies, such as LIT and Intravenous Immunoglobulin, do not significantly improve the live birth rate of RSA patients. Therefore, it is not suggested to conduct immunology therapies for RSA patients.

12.4.7 Environmental Factors

With the development of science and technology, medical workers pay more and more attention to the influence of environmental factors on embryo development. Antioxidants may be one of the factors to enhance the detoxification ability of human body and reduce the impact of bad environment on embryonic development. Dihydrotestosterone is the substrate of specific glucuronidation for UGT2B15 and UGT2B17 [53]. Antioxidants can activate the activity of GSTs and UGTs through Nrf2 approaches to enhance the metabolism [54]. Vitamin C can affect the variation of GSTs gene through combining with reactive oxide species [55]. Ikeda et al. [56] found that the levels of Vitamin C and E were higher in mice fed with sesamin and their mRNA levels of UGT1A and 2B in liver were also higher. In a recent study [57], the generation of ROS leads to DNA damage, activates DNA damage repair mechanism, and leads to the increase of DNA methylation level, while the antioxidant, N-Acetylcysteine (NAC) can inhibit the increase of DNA methylation level. In addition, the study shows that adding N-Acetylcysteine of appropriate concentration can significantly improve sperm survival index and prolong sperm survival time. Although there are not enough studies of the possible positive impact of antioxidants on embryonic development, more and more scholars have recognized that antioxidants may improve the impact of environment on embryonic development. The determination of metabolic enzyme activity may be an important method to diagnose the causes of recurrent spontaneous abortion, and antioxidants may become an important method to improve detoxification ability through enhancing the detoxification and metabolism activities of metabolic enzymes such as UGTs.

12.4.8 Male Factors

At present, more attention has been paid to factors and treatment of females in RSA research. However, studies have shown that occupational exposure, lifestyle (e.g., smoking, drinking), high sperm DNA fragmentation rate [58], and aneuploidy of sperm chromosomes [59] are possible causes of RSA. Therefore, it is suggested that the spouses of RSA patients should adjust their lifestyle and increase their physical

exercise appropriately before pregnancy. When sperm chromosomes are aneuploid, genetic counseling and evaluation were carried out.

References

1. Eggo RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, Nelen W, Peramo B, Quenby S, Vermeulen N, Goddijn M. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open. 2018;2018(2):4. https://doi.org/10.1093/hropen/hoy004.
2. Rai R, Regan L. Recurrent miscarriage. Lancet. 2006;368(9535):601–11. https://doi.org/10.1016/s0140-6736(06)69204-0.
3. Giudice LC. Environmental toxicants: hidden players on the reproductive stage. Fertil Steril. 2016;106(4):791–4. https://doi.org/10.1016/j.fertnstert.2016.08.019.
4. Sahoo T, Dzidic N, Strecker MN, Commander S, Travis MK, Doherty C, Tyson RW, Mendoza AE, Stephenson M, Dise CA, Benito CW, Ziadie MS, Hovanes K. Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits, and challenges. Genet Med. 2017;19(1):83–9. https://doi.org/10.1038/gim.2016.69.
5. Popescu F, Jaslow CR, Kutteh WH. Recurrent pregnancy loss evaluation combined with 24-chromosome microarray of miscarriage tissue provides a probable or definite cause of pregnancy loss in over 90% of patients. Hum Reprod. 2018;33(4):579–87. https://doi.org/10.1093/humrep/dey021.
6. Shahine L, Lathi R. Recurrent pregnancy loss: evaluation and treatment. Obstet Gynecol Clin N Am. 2015;42(1):117–34. https://doi.org/10.1016/j.ogc.2014.10.002.
7. Li J, Li X, Zhang S, Snyder M. Gene-environment interaction in the era of precision medicine. Cell. 2019;177(1):38–44. https://doi.org/10.1016/j.cell.2019.03.004.
8. Egerup P, Kolte AM, Larsen EC, Krog M, Nielsen HS, Christiansen OB. Recurrent pregnancy loss: what is the impact of consecutive versus non-consecutive losses? Hum Reprod. 2016;31(11):2428–34. https://doi.org/10.1093/humrep/dew169.
9. Silva TL, Carneiro PLS, Ambrosini DP, Lôbo RB, Filho RM, Malhado CHM. Genotype-environment interaction in the genetic variability analysis of reproductive traits in Nellore cattle. Livest Sci. 2019;230:103825. https://doi.org/10.1016/j.livsci.2019.103825.
10. Toth B, Jeschke U, Rogenhofer N, Scholz C, Würfel W, Thaler CJ, Makrigiannakis A. Recurrent miscarriage: current concepts in diagnosis and treatment. J Reprod Immunol. 2010;85(1):25–32. https://doi.org/10.1016/j.jri.2009.12.006.
11. Major J, Jakab MG, Tompa A. The frequency of induced premature centromere division in human populations occupationally exposed to genotoxic chemicals. Mutat Res. 1999;445(2):241–9. https://doi.org/10.1016/s1383-5718(99)00129-1.
12. Economopoulos KP, Sergentanis TN. GSTM1, GSTT1, GSTP1, GSTA1 and colorectal cancer risk: a comprehensive meta-analysis. Eur J Cancer. 2010;46(9):1617–31. https://doi.org/10.1016/j.ejca.2010.02.009.
13. Nonaka T, Takakuwa K, Tanaka K. Analysis of the polymorphisms of genes coding biotransformation enzymes in recurrent miscarriage in the Japanese population. J Obstet Gynaecol Res. 2011;37(10):1352–8. https://doi.org/10.1111/j.1447-0756.2011.01529.x.
14. Polimanti R, Piacentini S, Lazzarin N, Vaquero E, Re MA, Manfellotto D, Fuciarelli M. Glutathione S-transferase genes and the risk of recurrent miscarriage in Italian women. Fertil Steril. 2012;98(2):396–400. https://doi.org/10.1016/j.fertnstert.2012.05.003.
15. Zusterzeel PL, Nelen WL, Roelofs HM, Peters WH, Blom HJ, Steegers EA. Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. Mol Hum Reprod. 2000;6(5):474–8. https://doi.org/10.1093/molehr/6.5.474.
16. Meech R, Hu DG, McKinnon RA, Mubarokah SN, Haines AZ, Nair PC, Rowland A, Mackenzie PI. The UDP-glycosyltransferase (UGT) superfamily: new members, new functions, and

novel paradigms. Physiol Rev. 2019;99(2):1153–222. https://doi.org/10.1152/physrev.00058.2017.
17. Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Bélanger A, Fournel-Gigleux S, Green M, Hum DW, Iyanagi T, Lancet D, Louisot P, Magdalou J, Chowdhury JR, Ritter JK, Schachter H, Tephly TR, Tipton KF, Nebert DW. The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. Pharmacogenetics. 1997;7(4):255–69. https://doi.org/10.1097/00008571-199708000-00001.
18. Zhang Y, Hou J, Feng F, Li D, Jiang Q, Li X, Zhao Q, Li BA. Genetic polymorphisms in human UDP-glucuronosyltransferases 1A7 and the risk of gastrointestinal carcinomas: a systematic review and network meta-analysis. Oncotarget. 2017;8(39):66371–81. https://doi.org/10.18632/oncotarget.18675.
19. Petrenaite V, Öhman I, Ekström L, Sæbye D, Hansen TF, Tomson T, Sabers A. UGT polymorphisms and lamotrigine clearance during pregnancy. Epilepsy Res. 2018;140:199–208. https://doi.org/10.1016/j.eplepsyres.2018.01.011.
20. Reisinger TL, Newman M, Loring DW, Pennell PB, Meador KJ. Antiepileptic drug clearance and seizure frequency during pregnancy in women with epilepsy. Epilepsy Behav. 2013;29(1):13–8. https://doi.org/10.1016/j.yebeh.2013.06.026.
21. Li SN, Cao YF, Sun XY, Yang K, Liang YJ, Gao SS, Fu ZW, Liu YZ, Yang K, Fang ZZ. Hydroxy metabolites of polychlorinated biphenyls (OH-PCBs) exhibit inhibitory effects on UDP-glucuronosyltransferases (UGTs). Chemosphere. 2018;212:513–22. https://doi.org/10.1016/j.chemosphere.2018.08.040.
22. Wang F, Wang S, Yang K, Liu YZ, Yang K, Chen Y, Fang ZZ. Inhibition of UDP-glucuronosyltransferases (UGTs) by bromophenols (BPs). Chemosphere. 2020;238:124645. https://doi.org/10.1016/j.chemosphere.2019.124645.
23. Kapitulnik J. Bilirubin: an endogenous product of heme degradation with both cytotoxic and cytoprotective properties. Mol Pharmacol. 2004;66(4):773–9. https://doi.org/10.1124/mol.104.002832.
24. Servedio V, d'Apolito M, Maiorano N, Minuti B, Torricelli F, Ronchi F, Zancan L, Perrotta S, Vajro P, Boschetto L, Iolascon A. Spectrum of UGT1A1 mutations in Crigler-Najjar (CN) syndrome patients: identification of twelve novel alleles and genotype-phenotype correlation. Hum Mutat. 2005;25(3):325. https://doi.org/10.1002/humu.9322.
25. Itäaho K, Mackenzie PI, Ikushiro S, Miners JO, Finel M. The configuration of the 17-hydroxy group variably influences the glucuronidation of beta-estradiol and epiestradiol by human UDP-glucuronosyltransferases. Drug Metab Dispos. 2008;36(11):2307–15. https://doi.org/10.1124/dmd.108.022731.
26. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. Pharmacog J. 2003;3(3):136–58. https://doi.org/10.1038/sj.tpj.6500171.
27. Mackenzie PI, Miners JO, McKinnon RA. Polymorphisms in UDP glucuronosyltransferase genes: functional consequences and clinical relevance. Clin Chem Lab Med. 2000;38(9):889–92. https://doi.org/10.1515/cclm.2000.129.
28. Nagar S, Remmel RP. Uridine diphosphoglucuronosyltransferase pharmacogenetics and cancer. Oncogene. 2006;25(11):1659–72. https://doi.org/10.1038/sj.onc.1209375.
29. Ménard V, Eap O, Harvey M, Guillemette C, Lévesque E. Copy-number variations (CNVs) of the human sex steroid metabolizing genes UGT2B17 and UGT2B28 and their associations with a UGT2B15 functional polymorphism. Hum Mutat. 2009;30(9):1310–9. https://doi.org/10.1002/humu.21054.
30. Lewis BC, Mackenzie PI, Elliot DJ, Burchell B, Bhasker CR, Miners JO. Amino terminal domains of human UDP-glucuronosyltransferases (UGT) 2B7 and 2B15 associated with substrate selectivity and autoactivation. Biochem Pharmacol. 2007;73(9):1463–73. https://doi.org/10.1016/j.bcp.2006.12.021.
31. Stringer F, Scott G, Valbuena M, Kinley J, Nishihara M, Urquhart R. The effect of genetic polymorphisms in UGT2B15 on the pharmacokinetic profile of sipoglitazar, a novel anti-

diabetic agent. Eur J Clin Pharmacol. 2013;69(3):423–30. https://doi.org/10.1007/s00228-012-1382-7.
32. Hanioka N, Naito T, Narimatsu S. Human UDP-glucuronosyltransferase isoforms involved in bisphenol A glucuronidation. Chemosphere. 2008;74(1):33–6. https://doi.org/10.1016/j.chemosphere.2008.09.053.
33. Hunt PA, Lawson C, Gieske M, Murdoch B, Smith H, Marre A, Hassold T, VandeVoort CA. Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey. Proc Natl Acad Sci U S A. 2012;109(43):17525–30. https://doi.org/10.1073/pnas.1207854109.
34. Kong D, Xing L, Liu R, Jiang J, Wang W, Shang L, Wei X, Hao W. Individual and combined developmental toxicity assessment of bisphenol A and genistein using the embryonic stem cell test in vitro. Food Chem Toxicol. 2013;60:497–505. https://doi.org/10.1016/j.fct.2013.08.006.
35. Jiang HM, Fang ZZ, Cao YF, Hu CM, Sun XY, Hong M, Yang L, Ge GB, Liu Y, Zhang YY, Dong Q, Liu RJ. New insights for the risk of bisphenol A: inhibition of UDP-glucuronosyltransferases (UGTs). Chemosphere. 2013;93(6):1189–93. https://doi.org/10.1016/j.chemosphere.2013.06.070.
36. Nishikawa M, Iwano H, Yanagisawa R, Koike N, Inoue H, Yokota H. Placental transfer of conjugated bisphenol A and subsequent reactivation in the rat fetus. Environ Health Perspect. 2010;118(9):1196–203. https://doi.org/10.1289/ehp.0901575.
37. Tampal N, Lehmler HJ, Espandiari P, Malmberg T, Robertson LW. Glucuronidation of hydroxylated polychlorinated biphenyls (PCBs). Chem Res Toxicol. 2002;15(10):1259–66. https://doi.org/10.1021/tx0200212.
38. Wang D. The uridine diphosphate glucuronosyltransferases: quantitative structure-activity relationships for hydroxyl polychlorinated biphenyl substrates. Arch Toxicol. 2005;79(10):554–60. https://doi.org/10.1007/s00204-005-0671-7.
39. Net S, Delmont A, Sempéré R, Paluselli A, Ouddane B. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): a review. Sci Total Environ. 2015;515:162–80. https://doi.org/10.1016/j.scitotenv.2015.02.013.
40. Harris S, Wegner S, Hong SW, Faustman EM. Phthalate metabolism and kinetics in an in vitro model of testis development. Toxicol In Vitro. 2016;32:123–31. https://doi.org/10.1016/j.tiv.2015.12.002.
41. Ito R, Seshimo F, Miura N, Kawaguchi M, Saito K, Nakazawa H. Effect of sterilization process on the formation of mono(2-ethylhexyl)phthalate from di(2-ethylhexyl)phthalate. J Pharm Biomed Anal. 2006;41(2):455–60. https://doi.org/10.1016/j.jpba.2005.12.021.
42. Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum Reprod. 2007;22(10):2715–22. https://doi.org/10.1093/humrep/dem205.
43. Lovekamp TN, Davis BJ. Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. Toxicol Appl Pharmacol. 2001;172(3):217–24. https://doi.org/10.1006/taap.2001.9156.
44. Zheng B, Hu G, Yu J, Liu Z. Crigler-Najjar syndrome type II in a Chinese boy resulting from three mutations in the bilirubin uridine 5′-diphosphate-glucuronosyltransferase (UGT1A1) gene and a family genetic analysis. BMC Pediatr. 2014;14:267. https://doi.org/10.1186/1471-2431-14-267.
45. Du Z, Cao YF, Li SN, Hu CM, Fu ZW, Huang CT, Sun XY, Liu YZ, Yang K, Fang ZZ. Inhibition of UDP-glucuronosyltransferases (UGTs) by phthalate monoesters. Chemosphere. 2018;197:7–13. https://doi.org/10.1016/j.chemosphere.2018.01.010.
46. ACOG Practice Bulletin No.142. Cerclage for the management of cervical insufficiency. Obstet Gynecol. 2014;123(2 Pt 1):372–9. https://doi.org/10.1097/01.AOG.0000443276.68274.cc.
47. Cao Y, Sun H, Zhu H, Zhu X, Tang X, Yan G, Wang J, Bai D, Wang J, Wang L, Zhou Q, Wang H, Dai C, Ding L, Xu B, Zhou Y, Hao J, Dai J, Hu Y. Allogeneic cell therapy using umbilical cord MSCs on collagen scaffolds for patients with recurrent uterine adhesion: a phase I clinical trial. Stem Cell Res Ther. 2018;9(1):192. https://doi.org/10.1186/s13287-018-0904-3.

48. Glueck CJ, Smith D, Gandhi N, Hemachandra K, Shah P, Wang P. Treatable high homocysteine alone or in concert with five other thrombophilias in 1014 patients with thrombotic events. Blood Coagul Fibrinolysis. 2015;26(7):736–42. https://doi.org/10.1097/mbc.0000000000000276.
49. De Krom G, Arens YH, Coonen E, Van Ravenswaaij-Arts CM, Meijer-Hoogeveen M, Evers JL, Van Golde RJ, De Die-Smulders CE. Recurrent miscarriage in translocation carriers: no differences in clinical characteristics between couples who accept and couples who decline PGD. Hum Reprod. 2015;30(2):484–9. https://doi.org/10.1093/humrep/deu314.
50. Andrade C. Major malformation risk, pregnancy outcomes, and neurodevelopmental outcomes associated with metformin use during pregnancy. J Clin Psychiatry. 2016;77(4):e411–4. https://doi.org/10.4088/JCP.16f10789.
51. Empson M, Lassere M, Craig J, Scott J. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. Cochrane Database Syst Rev. 2005;2:Cd002859. https://doi.org/10.1002/14651858.CD002859.pub2.
52. Alijotas-Reig J, Ferrer-Oliveras R. The European registry on obstetric antiphospholipid syndrome (EUROAPS): a preliminary first year report. Lupus. 2012;21(7):766–8. https://doi.org/10.1177/0961203312440058.
53. Chouinard S, Yueh MF, Tukey RH, Giton F, Fiet J, Pelletier G, Barbier O, Bélanger A. Inactivation by UDP-glucuronosyltransferase enzymes: the end of androgen signaling. J Steroid Biochem Mol Biol. 2008;109(3-5):247–53. https://doi.org/10.1016/j.jsbmb.2008.03.016.
54. Bock KW. From differential induction of UDP-glucuronosyltransferases in rat liver to characterization of responsible ligand-activated transcription factors, and their multilevel crosstalk in humans. Biochem Pharmacol. 2011;82(1):9–16. https://doi.org/10.1016/j.bcp.2011.03.011.
55. Michels AJ, Hagen TM, Frei B. Human genetic variation influences vitamin C homeostasis by altering vitamin C transport and antioxidant enzyme function. Annu Rev Nutr. 2013;33:45–70. https://doi.org/10.1146/annurev-nutr-071812-161246.
56. Ikeda S, Abe C, Uchida T, Ichikawa T, Horio F, Yamashita K. Dietary sesame seed and its lignan increase both ascorbic acid concentration in some tissues and urinary excretion by stimulating biosynthesis in rats. J Nutr Sci Vitaminol. 2007;53(5):383–92. https://doi.org/10.3177/jnsv.53.383.
57. Berger ND, Stanley FKT, Moore S, Goodarzi AA. ATM-dependent pathways of chromatin remodelling and oxidative DNA damage responses. Philos Trans R Soc Lond Ser B Biol Sci. 2017;372:1731. https://doi.org/10.1098/rstb.2016.0283.
58. Tanaka T, Kobori Y, Terai K, Inoue Y, Osaka A, Yoshikawa N, Shimomura Y, Suzuki K, Minami T, Iwahata T, Onota S, Yamamoto A, Sugimoto K, Okada H. Seminal oxidation-reduction potential and sperm DNA fragmentation index increase among infertile men with varicocele. Hum Fertil. 2020;2020:1–5. https://doi.org/10.1080/14647273.2020.1712747.
59. Neusser M, Rogenhofer N, Dürl S, Ochsenkühn R, Trottmann M, Jurinovic V, Steinlein O, von Schönfeldt V, Müller S, Thaler CJ. Increased chromosome 16 disomy rates in human spermatozoa and recurrent spontaneous abortions. Fertil Steril. 2015;104(5):1130–7. https://doi.org/10.1016/j.fertnstert.2015.07.1160.

Part V
Effects of Environmental Factors on Fertility Preservation

Chapter 13
The Effects of Negative Elements in Environment and Cancer on Female Reproductive System

Jiangxue Qu, Yuehan Li, Shujie Liao, and Jie Yan

Abstract With the development of human society, factors that contribute to the impairment of female fertility is accumulating. Lifestyle-related risk factors, occupational risk factors, and iatrogenic factors, including cancer and anti-cancer treatments, have been recognized with their negative effects on the function of female reproductive system. However, the exact influences and their possible mechanism have not been elucidated yet. It is impossible to accurately estimate the indexes of female fertility, but many researchers have put forward that the general fertility has inclined through the past decades. Thus the demand for fertility preservation has increased more and more dramatically. Here we described some of the factors which may influence female reproductive system and methods for fertility preservation in response to female infertility.

Keywords Lifestyle-related risk factors · Occupational risk factors · Iatrogenic factors · Female fertility · Fertility preservation

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13.1 The Effects of Negative Elements in Environment on Female Reproductive System

13.1.1 Smoke and Intense Sport Taking

Cigarette smoke is consented to contain various chemical substances which may have harmful effects on human body, like polycyclic aromatic hydrocarbons and phenols, aldehydes. Studies on animals suggest that all of these ingredients exert toxic effects on female reproductive function and ovarian reserve. There have been plenty of studies which are dedicated to describing the possible connections between smoking and gynecological diseases.

It has been hypothesized that habitual smoking may contribute to the increased risk of premature ovarian failure (POI). POI can be manifested as an earlier age of menopause initiation or a decreased level of ovarian reserve. As proved by Chang et al. in 2007, smoking may contribute to an earlier age of menopause onset, with an OR rate of 1.82 [1.03–3.23] [1]. However, a cross-sectional study conducted in Italy in 2003 proposed an opposite conclusion, in which no significant association was found between the risk of POI and smoking [2]. On the other hand, as the serum level of anti-Müllerian hormone (AMH) is supposed to be applied as a predictive factor for ovarian function, Freour et al. tested the AMH level in 111 women treated for infertility, and discovered a significant decrease of AMH levels in patients who smoke (3.06 ± 1.68 mg/L versus 3.86 ± 1.92), while in 2016 Peck et al. analyzed the primordial follicle stock, which is seen as the most powerful and direct indicator for ovarian reserve, in 133 patients undergoing hysterectomy for benign diseases, put forward that no statistically important associations are found between follicle count and smoking [3, 4].

The results seem contradictory and confusing. In 2013, Sobinoff et al. conducted an experiment with mouse models exposed to smoke, with a result that smoke can cause decrease of ovarian follicular stock, and further elucidated the possible mechanisms of apoptosis and oxidative stress [5]. Oxidative stress is a pathophysiological activity which is characterized by an imbalanced relationship between pro-oxidant molecules and anti-oxidant molecules, and is discovered to contribute to pathogenesis of POI and infertility [6]. Though a systematic review reported that smoking is not statistically associated with female infertility (with an OR rate of 2.50, 95% CI.1.00, 6.30), studies showed that IVF pregnancy rate is decreased by almost a half in female smokers, as well as the deteriorated quality of donor eggs [7–9]. In 2017, with the assistance of high-throughput sequencing technique, 16s rRNA sequencing, Nelson et al. discovered that smoking may play an important role in altering vaginal microbiota into species that lacks protective Lactobacillus spp., which may subsequently contribute to the increased risk of bacterial vaginosis and malodor [10]. Cigarette smoking is unequivocally seen as an adverse environmental factor towards reproductive activities, and with the gradual completion of mouse models, more experiments will shed light on the exact mechanisms how smoke influences the female reproductive system [11].

Intense sport taking, both aerobic and anaerobic activities are believed to play a role in inducing an acute state of oxidative stress [12]. Moderate amounts of oxidative stress are necessary for human body to function well. Appropriate exercise intensity is found to be beneficial for physical fitness by decreasing malondialdehyde (MDA) and increasing both total anti-oxidant capacity (TAC) and interleukin-2 (IL-2) levels in formerly sedentary women [13].

13.1.2 Air Pollution

Air pollution has been under active investigation due to its increasingly notorious reputation for affecting various body functions through many mechanisms. There are four major pollutants that have been identified to exert harmful effects on human fertility: aerosols, nitrogen dioxide, sulfur dioxide, and carbon monoxide [14]. Aerosols are solid or liquid particles that suspend in the air, the diameter of which range from 2.5μm to 10μm, and is proved to be associated with decreased ovarian function, reduced fertility rate, and increased miscarriage rate. Nitrogen dioxide is transformed from nitric oxide with pro-oxidants, like O3 in the atmosphere, and potentially causes increased miscarriage rate and reduced live birth rate. It has been reported that perinatal exposure to nitrogen dioxide, particulate matter may cause adverse effects on nervous system development in fetus. As diesel engines produce diesel exhaust, and unlike gasoline engines, diesel engines are controlled by fuel supply other than air supply, which makes diesel exhaust contains great amount of polycyclic aromatic hydrocarbons (PAH) and heavy metal particles. These particles in particular matter are respirable and capable of causing negative impact on reproductive system, for PAH and heavy metal particles can generate reactive oxygen species that may lead to alterations in DNA. Noticeably, these particles may act as environmental endocrine disrupters, which means they are capable of imitating the functions of natural hormones and thus interfere with endogenous hormones' synthesis and production. Gonadal steroidogenesis and gametogenesis can be disrupted by environmental endocrine disrupters associated with estrogenic and testosterone activities.

13.1.3 Heavy Metals and Pesticides

Along with the rapid development of the industrial engineering technologies, invisible particles are getting more and more widespread in the environment we live and closely contact with human body. Heavy metals are discharged in wastewater produced by mining and metallurgy, machine manufacturing, which end up in spreading in rivers, lakes, and other sources where drinking water is extracted. These toxicants, which have long-term influence, are hard to detect at early stage. And when patients turn to clinical units because they have already suffered from

obvious symptoms, toxicants are much likely to have accumulated to an extraordinarily high level. Also, these toxicants exert adverse influence through various approaches, because they spread in the air we breathe, water we drink, and food we digest.

Cadmium is a multi-organ, multi-system toxicant with a long half-life period of 20–30 years, which has been proved to have obvious reproductive toxicity towards human body. It causes severe impairment in ovaries, including hemorrhage and atrophy of the organ, lessened mature oocytes, and increased number of atresia follicles. Besides, women with low endurance threshold toward cadmium may suffer from primary amenorrhea or POI before 40 years old. Epidemiology evidence shows that higher exposure level of cadmium is strongly related to higher incidence rate of pre-eclampsia [15], which is a severe pregnancy condition characterized by high blood pressure and threatens both maternal and fetal life.

Lead, also plumbum, Pb, has long been notorious for its toxicity towards many organs and tissues of human body, especially reproductive system and nervous system. It is reported that children are susceptible to lead exposure because it suppresses the development of the fetal nervous system by interfering with different processes including synapse formation, neuron migration and neuron-glia interactions [16]. For the female reproductive system, it is reported that lead exposure may cause a greater chance of the occurrence of recurrent pregnancy loss. The possible mechanisms lying behind this are hypothesized: lead has direct teratogenic influence towards the fetus, and it contributes to the dysfunction of the placental vascular. The adverse effects of lead on the female reproductive system are dose-dependent. However, even low serum level of lead may be extremely toxic, and it may accumulate in blood when aging. Place of residence should be taken into consideration when estimating the number of habitants recruited to evaluate the average concentration of lead in human body.

Mercury, universally used in human production activities, along with its naturally existing form in the nature, is widespread in the environment, and could be turned into methyl-mercury through the methylation process conducted by microorganisms. Furthermore, mercury can be bioaccumulated in living creatures. The concentration of mercury can be magnified in the food chain, and finally lead to the massive amount digested by human being. Contaminated aquatic products may cause methyl-mercury poisoning symptoms, including Minamata disease (symptoms covering spontaneous convulsions, loss of motor functions, and uncontrolled limb movements). Due to its chemistry characters, including small molecular weight, short carbon chain, non-ionizing feature, high fat solubility, methyl-mercury is easy to penetrate the placenta and blood–brain barrier, and consequently imposes irreversible intense toxic effects on the neuron system, especially in the developing brain. Besides, corresponding to different types of methyl-mercury exposure, various manifestations are seen due to female reproductive system damage. Acute poisoning symptoms may show infertility in female; subacute and chronic poisoning symptoms in pregnant women contain pregnancy loss and stillbirth; atypical toxication in pregnant women may result in giving birth to children with congenital Minamata disease.

13.2 The Effects of Cancer and Anti-Cancer Treatments on Female Reproductive System

Cancer, as a huge health problem, has affected more and more people. Its incidence continues to grow globally because of some cancer—causing habits and social factors such as unhealthy diet, smoking, and the growth and aging of the population [17, 18]. And in 2018, more than 1.27 million women aged from 15 to 44 were diagnosed with cancer. Due to the advances in early detection of cancer and improved treatment options, the survival rate of cancer increased obviously in the past few decades. The cure rate of cancer in childhood and adolescence has currently reached nearly 80%. From 2008 to 2012, the overall mortality rate of cancer in women is reduced by $>1.6\%$ per year and the ratio is expected to continue to fall significantly [19].

Howlader et al. showed that a partial percentage of cancer survivors are women at reproductive age [20]. A large number of women had not given birth to their first baby when being diagnosed with cancer [21]. And in order to improve the chance of survival after cancer diagnosis, there is a tendency in women with cancer to delay pregnancy [22]. Delayed pregnancy, cancer itself and its treatments have caused more cancer survivors to face the problem of establishing a family after finishing anti-cancer treatments. Researches present that more than 25% female cancer survivors are eager to bear children after the treatments [23, 24], and the proportion may be higher during the cancer diagnosis period [25, 26]. But the cancer process itself and its treatment are much likely to exert negative impacts on reproductive function, which makes the patients face a higher risk of infertility. Marriage and fertility are important aspects of a woman's life, which may significantly influence their happiness index. Infertility after anti-cancer treatments represents a serious problem for the patients' well-being, and it has negative effects on the quality of life of young survivors [27, 28]. Infertility can give patients tremendous mental stress and can destroy their social relations and disrupt their life plans in the future [27].

Oncofertility is a new integrated medical concept proposed by Woodruff in 2007. This new medical field is mainly aimed at young cancer patients after the survival of anti-cancer treatment, facing the status of reduced fertility and even infertility and proposes and implements a treatment strategy that can protect fertility during timely and effective anti-cancer treatment (http://www.fertilityprotect.cn/page/kecheng). The extent to which cancer and its treatment affects female fertility depends on the type of cancer, the age of the patients, and the specific therapies administered [29]. Cancer and its treatments can disrupt the function of the ovaries, the uterus, the hypothalamic-pituitary-ovarian axis, and other structures related to female fertility which are important parts of women's conception. What is the specific influence of cancer and anti-cancer treatment on female fertility? We will discuss about this topic in detail in the next part.

13.2.1 Influence of Malignant Cells on Female Fertility

Does the influence of malignant cells on female fertility really exist? Some studies showed that malignant tumors themselves can have adverse consequences for female fertility by having negative influence on the ovarian function [30–33]. Pal et al. reported firstly that malignancies have adverse effects of on oocyte quality [34], while Lubna Pal et al. were the first team to assess the effect of malignant tumors on ovarian function before performing treatments that are toxic to the gonads. Lubna Pal et al. showed that there were no differences in the number of oocytes retrieved between five patients with malignancies and 12 women of the same age who were infertile due to fallopian tube factors, however, patients in both groups reported rare matured oocytes, and there is a decreased fertilization rate in the cancer group. This implies that cancer may affect female fertility by impeding oogenesis. However, there are many studies, which include retrospective investigations involving larger populations, showed that no obvious differences are found in ovarian reserve and the number of oocytes between cancer patients and controls [35–45]. In addition, a prospective cohort study showed that after all patients have been treated by GnRH antagonist stimulation regimens, despite the comparable total number of oocytes that were retrieved, oocyte maturation was impaired [46]. Therefore, whether the malignancy itself has a potential negative impact on female fertility still needs to be continuously explored. Moreover, due to limited sample size, most studies have not explored the effects of different malignancies on female fertility based on specific type. However, as a result of different biological behaviors in different types of malignant tumors [47], it is necessary to consider the effects of various types of malignant tumors on ovarian function separately [48, 49]. For example, although breast cancer and hematological malignancies account for a large proportion of tumors in female patients of childbearing age, their effects on ovarian function are quite different. Breast cancer cells may not affect ovarian function, while in patients with hematological malignancies, there is a decrease in anti-Müllerian hormone and antral follicle count (Reduction of anti-Mullerian hormone and sinus follicle count indicate that ovarian function is impaired).

If malignant cells have a negative influence on female fertility, how is it affected? At present, it is not clear whether the underlying mechanisms include direct damage due to minimally invasive malignant cells, indirect impacts due to poor physical conditions and indirect impacts due to a paracrine interaction between tumor cells and ovarian tissue. It has been confirmed that mental stress generated after diagnosis of cancer destroys the normal function of hypothalamic-pituitary-gonadal axis [50]. The evidence, including the finding that cryopreserved ovarian tissue is potentially contaminated with tumor cells, shows that malignant cells may invade the ovarian, especially in leukemia patients [51]. However, it remains unknown that microinvasiveness is clearly associated with decreased ovarian function so that more evidence is needed.

There are some cytokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8) in patients with lymphoma that are possibly critical to follicular development and are related to lower levels of anti-Müllerian hormone (AMH) [52–54]. Nevertheless, no researches have confirmed that these cytokines are associated with lower ovarian function. Also, malignant cells may damage the function of granulosa cells [46]. And we can observe another histopathological phenomenon that offers evidence of direct impairment by malignancies. Raffaella Fabbri et al. detected many more cytoplasmic vacuoles in the oocytes of patients with Hodgkin's lymphoma (73.7%) than that in the control group (5.7%) [55]. Although the number of cases in this study is small, it can be seen that it is valuable to study the potential mechanism of the long-term role of malignant cells from the perspective of abnormal histological changes.

13.2.2 Malignant Tumors of Reproductive System

Female reproductive system malignancies include cervical cancer, uterine body cancer, ovarian cancer, and gestational trophoblastic tumor, etc. Cervical cancer, uterine body cancer, and ovarian cancer are three common female reproductive system malignancies. In recent years, with the changes in people's reproductive behavior, lifestyle and the surrounding environment, the risk of disease and death in the female reproductive system in China has been increasing.

It was found by R.M. Alvarez et al. that despite similar fertilization rates, compared with females with breast cancer or hematological malignancies, females with gynecological malignancies produce fewer mature oocytes during COS (controlled ovarian stimulation) [56]. The impact of reproductive system malignant tumors on female fertility depends on the site and kind of the cancer disease, the anti-cancer treatments protocol, and the age of the patient. For example, ovarian dysfunction, anovulation, infertility often occur in the younger group with endometrial cancer [57], similarly, pelvic radiotherapy (including external radiation and brachytherapy) in the advanced stage of cervical cancer and radical hysterectomy with salpingo-oophorectomy can cause female infertility. Perhaps many people think that reproductive system malignant tumors will affect ovarian function and even female fertility is unquestionable due to destruction of the reproductive system, but in fact, there is still no powerful evidence to prove this theory. More experiments are needed to demonstrate the impacts of reproductive system malignancies on female fertility.

13.2.3 Breast Cancer

Breast cancer is most common among women. In 2019, the proportion of women newly diagnosed with breast cancer is about 15% among all cancers (data from http://seer.cancer.gov/statfacts/html/breast.html.). Its mortality is declining and

survival rate has improved over the last 10 years. Fertility after anti-cancer treatment is essential for patients to consider which treatment regimens should be selected [58].

Letourneau JM et al. counted that, at the time of diagnosis of cancer, about 49%~65% of young females with breast cancer express a desire to have a baby after finishing anti-cancer treatments [59]. However, breast cancer patients are least likely to be pregnant in all female cancer survivors and have much lower fertility rates about 67% than ordinary people who are at the same age and education level [60]. The factors are as follows: on the one hand, the treatment of breast cancer will have a negative influence on female reproductive system. A large retrospective study indicated that breast cancer and its stage had no significantly negative influence on ovarian reserve function and response, but with the risk of biologically aggressive subtypes of tumors in young patients with breast cancer increasing [61–63], the subsequent treatments can cause gonadal dysfunction and infertility [64]. After confirming endocrine therapy in hormone receptor-positive breast cancer patients, it is often necessary to perform gonadotoxic chemotherapy and extend the treatment periods to 10 years, which may be the cause of infertility. On the other hand, women with BRCA1 or BRCA2 gene mutations not only have a higher risk of suffering from breast and ovarian cancer, but also increased risk of infertility. There is a hypothesis which says that BRCA germline mutations (BRCAm), which often occur in women with breast cancer, are related to accelerated follicular loss and early menopause. Females with BRCAm have a high risk of reduced ovarian reserve and infertility, whereas whether women who carry BRCA1/2 mutations have a reduced ovarian response remains controversial [65–67]. And with using chemotherapy (CT), CT-induced menopause may very likely occur in BRCA1m women due to lower ovarian reserve [68]. Therefore, breast cancer may cause serious adverse consequences for female fertility.

13.2.4 Hematological Malignancies

Common hematological malignancies mainly include various types of leukemia, multiple myeloma, and malignant lymphoma. There are approximately 0.5% of new cases diagnosed of Hodgkin Lymphoma, 4.2% of new cases diagnosed of Non-Hodgkin Lymphoma, 3.5% of new cases diagnosed of Leukemia in 2019 (http://seer.cancer.gov/statfacts/html/). Acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), Hodgkin lymphoma (HL), and non-Hodgkin lymphoma (NHL) are the most common types of hematological malignancies in girls and young women (https://www.lls.org/sites/default/files/file_assets/facts.pdf).

Hematological malignancies can have adverse influence on female fertility. On the one hand, hematological malignancies, themselves, have a negative impact on female fertility. There have been many studies demonstrating that lymphoma, one of the hematological tumors has a negative impact on ovarian function before radiotherapy and chemotherapy [53, 69–71], and it can be observed that despite using

similar amounts of gonadotropins, the baseline ovarian reserve of the tumor group is lower (evaluate through lower AMH and AFC) and the response to COH is poorer [53]. From the above studies, we know that women with HL have a low response to ovarian stimulation revealing some degree of follicle or oocyte dysfunction [53], and Hodgkin and non-Hodge Gold lymphoma is associated with decreased oocyte production [69]. In addition, van Dorp et al. found that impaired ovarian function also occurs in childhood leukemia [72]. Sklavos MM et al. showed that reduced ovarian reserve in females with hereditary bone marrow failure syndrome is related to the development of lymphoma and leukemia as well [73]. On the other hand, treatments of hematological malignancies also have an adverse effect on female fertility. The extent to which the treatment of hematological malignancies affects fertility in women depends on the type and period of the cancer, the dose of anti-tumor treatment, and the age of the patient [74]. Alkylation chemotherapy and total body irradiation (TBI) anti-cancer regimens are commonly used to treat teens and young females with hematological malignancies, particularly in cases of refractory and recurrent liver stem cell transplantation (HSCT), but these are invasive and gonadal toxic that can lead to infertility. Bone marrow transplantation (BMT) is also commonly used in women of lower reproductive age, and this treatment can produce a variety of reproductive adverse reactions such as ovarian failure, impaired uterine blood flow, and diminished uterine volume [75]. In summary, these treatments, including radiation therapy, chemotherapy, and bone marrow transplantation can lead to ovarian damage, uterine damage, and vaginal injury.

13.2.5 Childhood Malignancies

Childhood malignancies include childhood acute lymphoblastic leukemia (ALL), retinoblastoma, neuroblastoma, T cell childhood non-Hodgkin lymphoma (NHL), and Wilms' tumor. Although the incidence of malignant cancers in children is increasing year by year, the overall survival rates for children and teenagers diagnosed with cancer has been increasing due to improvements in cancer diagnosis and treatment and has now reached more than 80% [76]. Survival and infertility have become the two major concerns of children with malignant tumors that have shown to influence their quality of life causing huge mental stress [77].

Whether children's malignant tumors themselves have an impact on fertility requires further research and proof. Although Cassandra Roeca et al. found that female child cancer survivors who have delayed birth may have a higher risk of infertility, they also showed that childhood cancer survivors who recovered adequate ovarian function did not show an increase in ovarian reserve decline rate in early adulthood [78]. There is still no strong evidence of a direct relationship between childhood malignancies and infertility. However, there have been many studies showing that cancer treatment affects children's fertility. Childhood cancer treatment regimens have gonadotoxicity on different levels and may lead to a decline in fertility in the future. As Taylor et al. pointed out, infertility after childhood cancer

is a long-term problem affected by short-term treatment options [79]. Chemotherapy and radiotherapy are likely to damage the gonads to cause transient or life-long infertility [80–82]. Child malignant survivors are 20% less likely to become pregnant than unaffected siblings [83]. The extent of treatment affecting fertility depends on the age of patients and the type of treatments [84]. Patients with childhood malignant tumors who have received chemotherapy and intraperitoneal radiation have a significant increased risk of ovarian damage, decreased uterine volume, and preterm delivery and having offspring with low birth weight [85].

13.2.6 Influence of Anti-Tumor Treatments on Female Fertility

In the last decades, with the advances in available diagnostic tools and the improvement of treatment protocols, the overall survival rate of cancer has been significantly improved. Due to early diagnostic technique and successful adjuvant treatment, the number of young cancer survivors continues to increase and subsequently more and more young patients are facing fertility problems caused by cancer treatment so that the quality of life based on fertility has become the focus of women's attention. Anticancer treatments include surgery, radiotherapy, chemotherapy, endocrinotherapy, and immunotherapy. Unfortunately, although chemotherapy is improving, radiotherapy is more concentrated and surgical methods are more diverse, these treatments may all pose threats to female fertility including loss of fertility and sexual dysfunction. It is estimated that approximately 42% of women with cancer of childbearing age develop premature ovarian failure due to anti-cancer treatment [86]. The extent to which cancer treatment affects female fertility depends on the age of patients, the characteristic, duration, and dosage of treatments, and the sensitivity of each patient. Infertility is considered to be a serious long-term complication caused by cancer treatments, and it will have a negative impact on the quality of patients' life, patients' mental state and even their future plans [87, 88].

Cancer treatment may have adverse influence on female fertility for different reasons. Chemotherapy negatively affects female fertility by causing ovarian failure, damaging oocytes [89, 90], depleting the ovarian follicular reserve, and destroying hypothalamo-pituitary axis. For instance, some common anti-tumor drugs, such as cyclophosphamide and cisplatin, induce apoptosis of follicle cells through DNA breaks, which leads to follicle atresia [91]. Radiotherapy, especially in the abdomen and pelvis, can lead to ovarian destruction, reduced ovarian reserve, and so on [92]. And the impacts of chemotherapy and radiotherapy on female reproductive system are decided by many factors: the variety of drugs, size or location of the radiotherapy area, dosage, dose-intensity, method of administration, types of cancer, age, gender, and the pre-treatment fertility of the patient [93, 94]. Pelvic surgery has a critical effect on fertility as a consequence of adhesions and the removal of the ovaries, fallopian tube, and uterus as well [95]. Endocrine treatments are commonly

used in patients with breast cancer, which has direct and indirect impacts on fertility and ovarian function [82]. The direct impact that occurs only during therapy is as a result of damaged ovulatory and endometrial function, while the indirect impact is associated with delayed pregnancy, leading to ovarian aging [82]. However, there are few studies about the influence of immunotherapy on female reproductive system.

Neerujah Balachandren and Melanie Davies [96] divide the effects of treatments on fertility into three categories: (1) Female fertility after "high" gonadotoxic cancer therapies: therapies that at least 80% of the possibility will cause permanent amenorrhea are considered as highly gonadotoxicity [97]. These consist of hematopoietic stem cell transplantation requiring cyclophosphamide and/or total body irradiation (TBI), external radiation therapy to the ovaries or testes, or chemotherapy used to treat breast cancer in women over 40 years old [98]. (2) Female fertility after 'intermediate' gonadotoxic cancer therapies: therapies that 40%~60% of the possibility will cause amenorrhea are considered as intermediate gonadotoxicity, for example, adjuvant chemotherapy to treat 30~39 years old patients with breast cancer, step-by-step (second-line) chemotherapy for treating Hodgkin's lymphoma, and so on [98]. (3) Female fertility after 'low' gonadotoxic cancer therapies: only a few percent of the patients after 'low' gonadotoxic cancer treatments will have permanent amenorrhea. These include chemotherapy regimens consisting of first-line therapy for Hodgkin's lymphoma (ABVD treatment), and regimens to treat acute lymphoblastic and myeloid leukemia [99, 100].

In addition, anti-cancer treatments not only affect female fertility by directly damaging glandular structures and functions, but also indirectly cause female infertility through psychological influence. Most therapies may affect the physical and psychosocial aspects of the patient's sexual function. Anti-tumor treatments of reproductive system malignancy, particularly through radiotherapy and surgery, often alter the structure and function of the female reproductive tract and lead to decreased sexual desire [101, 102]. Fear, anxiety, and depression caused by anti-cancer treatments can reduce sexual desire, function, and frequency [103]. Therefore, we should not ignore the impact of psychological factors on fertility after anti-cancer treatments as well.

Next, we will specifically describe the influence of various anti-cancer treatments on female fertility.

13.2.7 Radiotherapy

Radiotherapy plays an important role in cancer treatments. In some cases, radiotherapy is the primary treatment for different malignancies in adolescents and young females (<45 years old), such as sarcomas, medulloblastomas, advanced cervical cancer, and Hodgkin's lymphomas [104, 105]. However, radiation has a gonadal toxicity that may causes long-term damage on the ovary, uterus, and hypothalamic-pituitary-gonadal axis. Even though the dysfunction of the reproductive organs

caused by radiotherapy can be temporary, recovery is usually unknown and in some patients the impairment can be permanent [106]. Radiotherapy in different parts will lead to different injuries. For example, total body and pelvic irradiation can affect both the ovary and the uterus, while skull irradiation may have an impact on the hypothalamic-pituitary-gonadal axis. Firstly, radiotherapy may result in ovarian failure. Radiotherapy is detrimental to ovarian reserve via impairing granulosa cells and causing follicle depletion [107]. Radiation also induces apoptosis by causing direct mitochondrial deoxyribonucleic acid (DNA) damage in oocytes and causes focal ovarian fibrosis by destroying blood vessels and impairing neovascularization. Secondly, radiotherapy causes damage to the uterine musculature and vascular system. Studies have shown that receiving radiotherapy due to childhood malignancies can lead to changes in uterine vascularization, reduced uterine volume and elasticity, uterine muscle fibrosis and necrosis, endometrial insufficiency and atrophy. The risk of spontaneous abortion, premature birth, and placental abnormalities will increase due to pelvic irradiation in childhood [108]. Thirdly, skull irradiation may cause damage to the hypothalamic-pituitary-gonadal axis, resulting in hormone secretion disorders. This is the result from a direct damage to H-P cells by radiotherapy [109]. And radiotherapy selectively damages hypothalamic neurons and pituitary cells, rather than general damage to the H-P axis [110, 111]. And the disorder of the pulsatile rhythm of FSH/LH may have negative impacts on fertility, sexual desire, and menstrual cycle. Moreover, hyperprolactinemia is another potential outcome of radiotherapy and is often associated with a decrease in the inhibitory neurotransmitter dopamine levels. Mild to moderate increases in PRL levels after low-dose radiation occasionally lead to amenorrhea in females and delayed puberty in children [112].

The effect of radiotherapy on female fertility depends on some factors, including the age of patients, radiation area, type, dosage, and duration of the therapy [113]. Firstly, different influencing factors have different effects on the ovaries. Researches have indicated that the doses of radiation varying from <2 Gy to 4 Gy can destroy up to 50% of human follicles [114], whereas 25 to 50 Gy radiation can cause one-third of young females and almost all females over 40 years old infertile [115–117]. Girls exposed to radiation >5 Gy have a 50% reduced chance of conception, while girls exposed to radiation >10 Gy have an increased risk up to 80% [118]. Moreover, premature ovarian insufficiency (POI) is related to more than 10 Gy irradiation on the ovaries [116]. Ovarian failure is associated with radiation dose. Wallace has shown that ovarian failure occurs at birth with the dosages of 20.3 Gy, at 10 years with the dosages of 18.4 Gy and at age 20 with the dosages of 16.5 Gy [119]. And ovarian impairment is associated with the age when receiving radiotherapy. The ovary is more resistant to radiation early in life, as the age increases, the dosage that causes damage is reduced [114]. After pre-pubertal girls receive 10–15 Gy radiation, the risk of amenorrhea is 30–70%, and the doses that cause post-pubertal girls to have amenorrhea are as low as 5–10 Gy [120]. The distance from the location of the radiation to the ovary and whether it is fractionated is also an influencing factor. Single radiation is more toxic than multiple divided doses that reach the same or even higher cumulative dose [121]. When 40 Gy is

transmitted to the cervix, the scattered radiation transferred to the ovary at the field boundary of 3~4 cm is 2~28 Gy [122]. Secondly, different radiation doses have different effects on the uterus. In adults, Total Body Irradiation (TBI) of 12 Gy may cause uterine damage, while radiation dosages of >25 Gy concentrated in the uterus can cause irreversible impairment to the child's uterus [123]. Radiation doses that can cause damage to the uterus are still under investigation. In 2014, Teh et al. have recommended that adults >45 Gy and childhood >25 Gy should be advised to avoid pregnancies [124]. Thirdly, different influencing factors have different effects on the hypothalamus-pituitary-gonadal axis. 18~24 Gy activates the hypothalamus and promotes its secretion of gonadotropin-releasing hormone (GnRH), which affects the production of gonadotropins in the pituitary gland and stimulates the ovaries, leading to precocious puberty [125]. Radiation at a dose of 40~50 Gy or even 30 Gy may directly damage the hypothalamus-pituitary-gonadal axis. Radiotherapy with a radiation dose >30 Gy to the hypothalamus and pituitary can reduce the likelihood of pregnancy by 40% [118]. Dosages of 40 Gy or higher can cause hyperprolactinaemia, which may lead to amenorrhoea as well [126, 127]. Radiotherapy damage to the hypothalamus is also related to age that immature hypothalamus is more radiosensitive than mature one [127].

13.2.8 Chemotherapy

It has been confirmed that chemotherapy has negative influence on the female fertility. Chemotherapy can damage the ovaries and the extent of ovarian damage and the potential for infertility is decided by the type and doses of the drugs, the age and gonadal conditions before treatment of the patients [128, 129]. It is known that chemotherapy can cause a decrease in ovarian reserve and subsequently bring out early menopause [130]. Reduced ovarian reserve is due to a decline in the quantity of follicles or even a complete lack of follicles and fibrosis caused by chemotherapy. Chemotherapy also have adverse effects on ovarian stromal or vascular function. Histological studies have shown that chemotherapy has an indirect impact on stromal cells [131]. After administering doxorubicin, a dramatic decrease in the blood volume in the ovary and ovarian vasospasm were presented by monitoring the blood flow in vivo [132]. There are also other mechanisms by which chemotherapy can cause ovarian damage, such as vascular injury and focal fibrosis of the ovarian cortex. In addition, besides the loss of fertility, it leads to the so-called climax syndrome. Chemotherapy can make female patients suffer from subjective symptoms such as loss of libido and objective accessory symptoms such as genital atrophy, vaginal atrophy, and dyspareunia [133–137]. Therefore, the effect of chemotherapy on female fertility is multifaceted.

From many studies, chemotherapeutic drugs have been divided into three types of risk on the basis of their gonadotoxicity [138, 139]: high risk-Alkylating drugs (cyclophosphamide, busulphan, chlorambucil, procarbazine, melphalan, ifosfamide, chlormethine); medium risk-Platinum drugs (cisplatin, carboplatin); anthracycline

antibiotics (adriamycin [doxorubicin]); taxoids (docetaxel and paclitaxel); low risk-Vinca plant alkaloids (vincristine and vinblastine); anthracycline antibiotics (bleomycin), antimetabolites (methotrexate, 5-fluorouracil, 6-MP [mercaptopurine]). And also different chemotherapeutic drugs negatively affect the ovaries through different mechanisms: Alkylating drugs-forming covalent bonds between DNA strands, interfering with cleavage during DNA replication and demaging cell division, thereby having a direct negative effect on oocytes [140]; platinum-based compounds-although existing researches indicate that it is not specifically toxic to human primordial follicles, there is a research indicating that Cisplatin can cause follicular atresia by DNA break-induced apoptosis of follicular cells [141]; antimetabolites and Vinca alkaloids-there is no DNA impairment to human follicles so that there is no gonadotoxicity; Anthracyclin antibiotics-Doxorubicin causes DNA chains breaks P63-mediated apoptotic death in human primordial follicles [140]; other agents, such as docetaxel, demage ovarian-somatic cells with secondary oocyte death [142]. In short, chemotherapy can impair the structures and functions of oocytes and granulosa cells in the ovarian follicle [143].

13.2.9 New Therapeutic Techniques

New therapeutic techniques include new surgical treatments, new chemotherapy drugs, targeted therapy, and interventional, biological, genetic, photodynamic, and radioisotope treatments.

Although there are few studies about the effects of new drugs and targeted therapies on female reproductive system, a study has shown that the anti-angiogenic agent bevacizumab may cause ovarian failure to 34% colorectal cancer patients compared to the group given the same treatment except using bevacizumab. In addition, tyrosine kinase inhibitors have teratogenic effects in laboratory animal models so that being pregnancy during treatment is not recommended in human [144]. But the delayed childbearing may lead to infertility. Therefore, new therapeutic techniques may also have a negative impact on female reproductive system, but more studies are needed to prove it.

13.3 Fertility Preserving Strategies and Technologies

Developments in cancer treatments have led to an increased survival rate and a longer life span in female cancer patients. For patients who are in their age of fertility, side effects of chemotherapy and radiotherapy, may lead to ovarian dysfunction, which result in early menopause and infertility. Thus the demand for fertility preservation has increased dramatically.

In 2009, Roger et al. systematically retrospected the development of fertility preservation in the past decade, and proposed that, “Fertility preservation” can be

defined as "through surgery, medical or laboratory procedures, before the end of natural reproductive life, retain the potential for genetic parenting of adults or children at risk of fertility [145]". Detrimental influences on fertility which result from cancer or its treatment protocol should be acquainted by the patient before the beginning of the treatment [129]. According to the guideline from American Society of Clinical Oncology and revised in 2013, potential gonadotoxic effects of cancer treatments deserve to be discussed with a group of cross-disciplinary experts including oncologists, reproductive specialists, and psychosocial providers [146], which is particularly helpful for the panic patients to ease the stress and make optimal decision considering the future possibility of conceiving. Although some fertility preservation methods have been validated in the past decade, many health-care workers are unfamiliar with the rapid advancements in fertility preservation [147]. Because of the emotional shock at the cancer diagnosis, and complex investigations and procedures, only a small number of patients are provided with the counseling on fertility preservation before the beginning of the treatments [148]. Current evidence suggests that psychosocial workers' assistance in the decision-making process for patients searching for fertility preservation helps them to ease the stress and to make morally and ethically acceptable choices [149–151], and thus are of great importance to the patients and their families [152].

Though various fertility preservation options are currently presented, some are regarded as experimental, and physicians and oncologists need to deliberately choose regiments for each patient according to patient cancer type, maturity level and her wish whether to become a mother.

13.3.1 Fertility Preserving Surgery

For fertility preservation, surgical treatments include fertility-sparing surgery and ovarian transposition [153]. Fertility-sparing surgery (i.e. trachelectomy) is safely performed without increasing the total mortality rate in gynecologic cancer patients like early-staging cervical cancer and patients with stage IA and IC epithelial ovarian cancer, and it helps to preserve the patient's potential to bear children [154–156]. For cervical cancer patients, it has been presented that different types of fertility preservation procedures are used considering the surgical procedures and the extent of para-cervical resection, and radical vaginal tracheostomy (VRT) and abdominal radical trachelectomy (ART) have similar oncology results for tumors <2 cm in length and are now known as safe procedures [157]. However, Down-staging tumors larger than 2 cm via neoadjuvant chemotherapy is still an experimental procedure. Neoadjuvant chemotherapy, a concept which was first adopted at Yale in 1979, is adapted before the surgery for women with medical co-morbidities and/or poor performance statuses that would significantly limit aggressive cytoreductive surgery [158]. There are various combined chemotherapy administrations for breast cancer, and according to Chinese Anti-Cancer Association it can be divided into four major kinds: regimens centered with anthracyclines, like CAF regimen (C stands for

cyclophosphamide; A for adriamycin; F for fluorouracil); regimens consisting of taxane and anthracyclines; sequential regimens including taxane and anthracyclines; and other regimens, like PC (P stands for paclitaxel taxol, C for carboplatin) [159].

Ovarian transposition is traditionally carried out for patients who are undergoing pelvic radiotherapy to preserve ovarian functions [160]. Live births have been reported after ovarian transposition [161], yet because of the scatter of radiation, this process is not always successful [147], also ovarian transposition will not spare the uterus free from adverse effect caused by radiation [160].

13.3.2 Fertility Preserving Medications

Hormone treatment, including GnRH agonist (gonadotropin-releasing hormone agonist, GnRH-a,), progesterone, anti-estrogen, aromatase inhibitors, by downregulating the susceptivity of the primordial follicles, aiming at protecting follicles against damages caused by radiotherapy and chemotherapy. GnRH-a is applied before chemotherapy, which is a conventional medical treatment for hematologic malignancies, as well as breast, ovarian, and cervical cancers. By suppressing the hypothalamus-pituitary-ovary axis, GnRH-a leaves follicles of little susceptivity towards ovarian-toxic agents in the long term, which helps to preserve follicle storage and ovarian functions [162]. However, no definitive evidence ensures its validity in fertility preservation [163, 164], the application of GnRH analogs is regarded experimental and controversial [163, 165, 166]. A study showed little beneficial effects of GnRH analogs on the menstruation recovering [165]; while a meta-analysis which included 24 months of follow-up after the study of one of GnRH analogs, Zoladex, proved it may have no beneficial effect on either maintenance of menstrual cycles or fertility [167]. It is still in demand for further research to ascertain whether to use GnRH in fertility preservation. Studies which analyzed the fertility outcomes in women with endometrial hyperplasia or early stage, low grade carcinoma with high-dose progestin therapy have proved its safety and feasibility in fertility preservation [168, 169], and progestin can be used alone as a primary fertility-sparring chemotherapy in premenopausal women with endometrial carcinoma [170]. It has long been recognized that breast cancer is hormone-dependent, and which is positively affected by the down regulation of serum estrogen or progesterone concentration. It has been reported that the use of aromatase inhibitors as adjuvant hormone therapy for gonadotropins (such as follicle stimulating hormone, FSH) can reduce the increasing rate of serum estradiol concentration [171], and provide a possibility to reduce the negative impacts of the ovarian stimulation procedures on breast cancer patients who demanding fertility preservation [172].

13.3.3 Fertility Cryopreservation

13.3.3.1 Embryo or Oocytes Cryopreservation/Vitrification

Developed on the basis of systematic reviews, guidelines recommend cryopreservation of embryos or oocytes to be established and validated practice for female cancer patients to ensure future fertility [146]. Embryo cryopreservation has been used since the early stage of assisted reproductive technology, and for post-pubertal women, conventional embryo or oocyte cryopreservation yields the highest live birth rate [173]. With a long-term partner and without other limitations, female about to undergo cancer treatments should be provided with the opportunity to cryopreserve their embryos, which is embraced with the highest success rates comparing to other fertility preservation methods [174].

A recent study which included 2157 women, who experienced the first in vitro fertilization cycle, experienced fresh-embryo transfer or embryo cryopreservation, and then were given frozen-embryo transfer, verified that transferring embryos that has been cryopreserved and then thawed, will get nearly the same live birth rate as transferring fresh embryos [175, 176]. On the other hand, Chen et al. [177] suggest that in women with the polycystic ovary syndrome, frozen-embryo transfer tend to have a higher rate of live birth compared with fresh-embryo transfer. And the risk of ovarian hyperstimulation syndrome is lower and the risk of pre-eclampsia after the first transfer is higher as well. As for people who do not have a male partner at the time in need for fertility preservation process, cryopreservation for mature oocytes can act as an alternative.

Cryopreservation use two major types of methods, slow freezing and vitrification [147]. Vitrification allows the solidification of the cells and the extracellular substances into a glass-like state without the formation of ice. Recently, a systematic review and meta-analysis have demonstrated that vitrification is superior to slow freezing due to live birth rate, and suggests that with regard to clinical outcomes, laboratories that continue to use slow freezing should consider transition to the use of vitrification for cryopreservation [178].

Because of the different situation between cancer patients and patients who wish to retain fertility with benign conditions, some points worth noticing. First, prepubescent cancer patients are unable to follow the oocytes retrieving process due to sexual immaturity [160, 179]. Second, controlled ovarian stimulation, which is conventionally carried out as a process in cryopreservation, may add estradiol to circulatory system, and is risky for sexual-hormone-sensitive cancers like breast and endometrial cancer [180]. Third, oocyte or embryo cryopreservation demands for at least 2 weeks of time before the commencement of the cancer therapy, and it is not advisable if the postpone will jeopardize the outcome of the patient [147].

13.3.3.2 Ovarian Tissue Cryopreservation

For pre-pubertal girls, the fertility preservation options are limited. As fertility outcomes after ovarian transposition are uncertain [181], ovarian tissue cryopreservation is the only method of fertility preservation [146, 147, 160, 179, 180, 182, 183]. Also, ovarian tissue cryopreservation does not involve the process of controlled ovarian stimulation, which makes it one of the most optimal choices for hormone-sensitive cancers like breast cancer patients. Hopefully, cryopreservation of ovarian tissue could not only supply oocytes but also probably help young patients regain ovarian endocrine functions. Emerging data indicate that obtaining multiple biopsies from one ovary does not deteriorate the future hormone production [184]. However, ovarian tissue cryopreservation is regarded as experimental, for on most case a secondary surgery of reimplantation of ovarian tissue is needed, which may lead to recurrence of the primary malignant disease. Four separated studies, which used polymerase chain reaction (PCR), flow cytometry analysis, proved that ovarian tissue cryopreserved from patients with leukemia may contain leukemic cells in more than 50% of cases, and result in recurrence of the disease [185–188]. Soares et al. [189] evaluated a purging procedure, involving three times of washing, proved effective for eliminating leukemic cells in ovarian tissue while maintaining the follicles viability. The procedure indicates a promising technique for follicle isolation process that can effectively be followed with ovarian tissue cryopreservation. On the other hand, ovarian capillaries are easily damaged, and more than half of the dormant follicles are lost during the ischemic period [160]. The combination of ovarian tissue cryopreservation with immature oocyte collection from the tissue followed by in vitro maturation of the oocytes has been reported as a promising new approach [180]. And the combination of vitrification of oocytes and cryopreservation of ovarian tissue yields a live birth rate of 50%~60% [148].

Considering that vitrification has gradually replaced the slow-freezing process of embryos and oocytes cryopreservation, it deserves discussing that whether vitrification is also better in the case of ovarian tissue cryopreservation. Slow freezing of ovarian cortex is still applied in most centers, while vitrification of ovarian tissue is under exploration, and the first live birth after transplantation of vitrified-thawed ovarian tissue was reported in 1999 [190]. To date, vitrified ovarian tissue has resulted in a few live birth reports, and it is demonstrated recently that in terms of the proportion of the intact follicles and the clinical outcomes, vitrification is not inferior to slow-freezing techniques [191, 192]. Recently a systematic review and meta-analysis showed that vitrification was associated with significantly less primordial follicular DNA damage and better preservation of stromal cells [193].

13.3.4 Other Newly-Developed Technologies

Several novel concepts may hold possibility to contribute to protect reproductive function [148, 194], including artificial ovary [195, 196], ovarian stem cells [197], preventive strategies which can mitigate gonadal-toxic effects [198].

Artificial ovary is conceived that primordial follicles are retrieved and are transferred on a framework, and after transplanting this artificial organ, obtaining mature oocytes become a possible. In a mouse model, it was observed that isolated murine follicles survived and grew inside a fibrin scaffold, and for the first time it demonstrated that preantral follicles can successfully survive and develop after grafting when encapsulated in fibrin matrices [195]. It is promising that artificial ovary can eliminate malignant cells and in the meantime maintain reproductive functions after a simple three-step purging procedure [189, 199].

For a long time, it had been believed that ovarian germ cells are no longer produced after birth in some species including human beings, mice, and pigs. And it was noteworthy when reports first demonstrated in 2004 that oogonial stem cells (OSCs) can actually be cultured into oocytes under certain conditions [200, 201], and it is under discovery that whether the oocytes cultured can be combined with support cells to form mature follicle which can consequently be fertilized [202]. A double labeling method adapting both proliferating cell markers, which include 5-Bromo-2-Deoxyuridine (BrdU), and germ cell markers are used to specifically identify proliferating ovarian stem cells [203]. Acting as a specific marker for germ cells, DEAD box polypeptide 4 (Ddx4), also referred to as Mouse Vasa homolog (MVH) was reported [204]. In 2004, a study used MVH and BrdU marks to identify the OSCs in a neonatal mouse model [200]. Also, studies reported that mouse OSCs, which had been labeled with green fluorescent protein (GFP) and then cultured in vitro, were able to induce GFP-positive fertilized oocyte after being transplanted back to the ovaries [205, 206]. Recently, female primordial germ cell-like cells in mice, which were derived from embryonic stem cells and subsequently cultured in vitro and transplanted back to the mice ovaries, are found to produce meiotically competent oocytes that could be fertilized to produce offsprings [207]. Also, studies reported successful induced maturation process from stem cell to sperm in male mice [208]. The possibility of in vitro-derived germ cells may provide patients, whose ovarian reservation are damaged by cancer treatment, with a greater chance of fertility preserving regardless of their puberty status, which demands further exploration. However, ovarian stem cells treatments, which involving ovarian stem cells cryopreservation and reimplantation, or growing 'egg precursor' cells in vitro and transplanting them back to the ovary, are still considered preclinical which demands for further exploration [203].

Encapsulation of the chemotherapeutic agents has made it possible that chemotherapy drugs be targeted-delivered to the solid tumor without damaging the function of reproductive system [209]. This method, which encapsulates chemotherapeutic drugs in nanoparticles, is based on the difference of vascular densities between solid tumor and normal tissues [210]. Through the years, many delivery systems have

been established, including viral delivery agents [211, 212], non-viral delivery agents (nano-materials with inorganic, synthetic polymeric, biological or hybrid composition) [213, 214], but due to their drawbacks, like unpredictable viral cytotoxicity, and unsatisfactory delivery efficiency in synthetic materials [215], endogenous cellular components are developed. Two delivery agents are now the most popular nano-carriers: exosomes and (apo) ferritin [216–220]. Considering that these two biomolecules actually exist in human body, they are of benefit to act as drug delivery agents, which can efficiently decrease the adverse effects of chemotherapy. Recently, a study after reviewing the mechanisms of action and toxicity of doxorubicin, reported a liposome-based drug-delivery system, which proved to be promising to reduce toxicity [221]. Protective methods are applied to mitigate the systemic side effects caused by chemotherapy or radiotherapy, which shows promising effects.

For female cancer patients, fertility preservation should be counseled before they undergo treatments. Currently, the American Society of Clinical Oncology (ASCO) and the American Society of Reproductive Medicine (ASRM) have published guidelines, providing specific strategies for clinical physicians to tailor fertility preservation protocols for cancer patients. ASCO recommends that health providers (including oncologists) provide the patients with information regarding potential threats to fertility early in the cancer treatment process, and that sperm, embryo, as well as oocyte cryopreservation be regarded as standard practice. Depending on the patient's age, puberty status, or parent own wish to conceive, fertility preservation methods, including cryopreservation of embryos, oocytes, ovarian tissue, and ovary transposition surgery, help to protect the functions of the reproductive system from being damaged by cancer or its treatment. Novel approaches, like artificial ovary, oogonial stem cells, and targeted therapy, are showing experimental potentials for preserving ovarian functions or rebuilding ovarian reservation, while still in demand for further validation of clinical evidence. Ensuring the safety and efficacy of fertility preservation techniques, and offering more cancer survivors at risk of reproductive complications, should be implemented in the near future.

13.4 Summary

There are still limited figure on the effect of negative elements in environment, cancer and anti-cancer treatments on female fertility. For the vast majority of negative elements in environment, cancer and anti-cancer treatments, the actual risk of infertility has not been determined. More research is needed to verify the influence of negative elements in environment, cancer and anti-cancer treatment on female reproductive system and fertility preservation. Moreover, it is also important to formulate a reasonable treatment plan such as fertility preserving technologies based on these studies. Now it is found that fertility preservation is a common practice for males, but for females, it is not commonly used and still in the experimental stage before puberty. Thus, protecting female fertility to deal with

these bad effects and improve their quality of life and long-term expectations should be an important concern.

13.5 Conclusion

The influence of environmental factors and iatrogenic factors on female fertility and its mechanism are gradually understood by people. This is an emerging interdisciplinary field and deserves full cooperation and in-depth research by scientists and clinicians.

References

1. Chang SH, et al. Premenopausal factors influencing premature ovarian failure and early menopause. Maturitas. 2007;58(1):19–30.
2. Progetto Menopausa Italia Study Group. Premature ovarian failure: frequency and risk factors among women attending a network of menopause clinics in Italy. BJOG. 2003;110(1):59–63.
3. Freour T, et al. Active smoking compromises IVF outcome and affects ovarian reserve. Reprod Biomed Online. 2008;16(1):96–102.
4. Peck JD, et al. Lifestyle factors associated with histologically derived human ovarian non-growing follicle count in reproductive age women. Hum Reprod. 2016;31(1):150–7.
5. Sobinoff AP, et al. Scrambled and fried: cigarette smoke exposure causes antral follicle destruction and oocyte dysfunction through oxidative stress. Toxicol Appl Pharmacol. 2013;271(2):156–67.
6. Agarwal A, et al. The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol. 2012;10:49.
7. Waziry R, et al. The effects of waterpipe tobacco smoking on health outcomes: an updated systematic review and meta-analysis. Int J Epidemiol. 2017;46(1):32–43.
8. Hughes EG, et al. Cigarette smoking and the outcomes of in vitro fertilization: measurement of effect size and levels of action. Fertil Steril. 1994;62(4):807–14.
9. Soares SR, et al. Cigarette smoking affects uterine receptiveness. Hum Reprod. 2007;22 (2):543–7.
10. Nelson TM, et al. Cigarette smoking is associated with an altered vaginal tract metabolomic profile. Sci Rep. 2018;8(1):852.
11. Meldrum DR, et al. Aging and the environment affect gamete and embryo potential: can we intervene? Fertil Steril. 2016;105(3):548–59.
12. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. Dyn Med. 2009;8:1.
13. Leelarungrayub D, et al. Six weeks of aerobic dance exercise improves blood oxidative stress status and increases interleukin-2 in previously sedentary women. J Bodyw Mov Ther. 2011;15(3):355–62.
14. Checa Vizcaino MA, Gonzalez-Comadran M, Jacquemin B. Outdoor air pollution and human infertility: a systematic review. Fertil Steril. 2016;106(4):897–904.e1.
15. Rosen EM, et al. Environmental contaminants and preeclampsia: a systematic literature review. J Toxicol Environ Health B Crit Rev. 2018;21(5):291–319.

16. Dorea JG. Environmental exposure to low-level lead (Pb) co-occurring with other neurotoxicants in early life and neurodevelopment of children. Environ Res. 2019;177:108641.
17. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
18. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65:87–108.
19. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62:10–29. https://doi.org/10.3322/caac.20138.
20. Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, et al., editors. SEER cancer statistics review, 1975–2012. Bethesda, MD: National Cancer Institute; 2015. http://seer.cancer.gov/csr/1975_2012/, based on November 2014 SEER data submission, posted to the SEER web site
21. Martin J, Hamilton B, Sutton P, Menacker F, Kirmeyer S. Births: final data for 2004. Natl Vital Stat Rep. 2006;55:1–101.
22. Juan B, Gratacós E. Delayed childbearing: effects on fertility and the outcome of pregnancy. Fetal Diagn Ther. 2011;29(4):263–73.
23. Armuand GM, Wettergren L, Rodriguez-Wallberg KA, Lampic C. Desire for children, difficulties achieving a pregnancy, and infertility distress 3 to 7 years after cancer diagnosis. Support Care Cancer. 2014;22(10):2805–12.
24. Hulsbosch S, Koskas M, Tomassetti C, De Sutter P, Wildiers H, Neven P, D'Hooghe T, Amant F. A real-life analysis of reproductive outcome after fertility preservation in female Cancer patients. Gynecol Obstet Investig. 2018;83(2):156–63.
25. Gorman JR, Malcarne VL, Roesch SC, Madlensky L, Pierce JP. Depressive symptoms among young breast cancer survivors: the importance of reproductive concerns. Breast Cancer Res Treat. 2010;123(2):477–85.
26. Huyghe E, Sui D, Odensky E, et al. Needs assessment survey to justify establishing a reproductive health clinic at a comprehensive cancer center. J Sex Med. 2009;6(1):149–63.
27. Tschudin S, Bitzer J. Psychological aspects of fertility preservation in men and women affected by cancer and other life-threatening diseases. Hum Reprod Update. 2009;15:587–97. https://doi.org/10.1093/humupd/dmp015.
28. Niemasik EE, Letourneau J, Dohan D, Katz A, Melisko M, Rugo H, Rosen M. Patient perceptions of reproductive health counseling at the time of cancer diagnosis: a qualitative study of female California cancer survivors. J Cancer Surviv. 2012;6:324–32. https://doi.org/10.1007/s11764-012-0227-9.
29. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, Beck LN, Brennan LV. Oktay K; American Society of Clinical Oncology. J Clin Oncol. 2006;24:2917–31.
30. Agarwal A, Said TM. Implications of systemic malignancies on human fertility. Reprod Biomed Online. 2004;9:673–9.
31. Quintero RB, Helmer A, Huang JQ, et al. Ovarian stimulation for fertility preservation in patients with cancer. Fertil Steril. 2010;93(3):865–8.
32. Domingo J, Guillén V, Ayllón Y, et al. Ovarian response to controlled ovarian hyperstimulation in cancer patients is diminished even before oncological treatment. Fertil Steril. 2012;97(4):930–4.
33. Friedler S, Koc O, Gidoni Y, et al. Ovarian response to stimulation for fertility preservation in women with malignant disease: a systematic review and meta-analysis. Fertil Steril. 2012;97(1):125–33.
34. Pal L, Leykin L, Schifren JL, et al. Malignancy may adversely influence the quality and behaviour of oocytes. Hum Reprod. 1998;13(7):1837–40.
35. Knopman JM, Noyes N, Talebian S, Krey LC, Grifo JA, Licciardi F. Women with cancer undergoing ART for fertility preservation: a cohort study of their response to exogenous gonadotropins. Fertil Steril. 2009;91:1476–8.

36. Quintero RB, Helmer A, Huang JQ, Westphal LM. Ovarian stimulation for fertility preservation in patients with cancer. Fertil Steril. 2010;93:865–8.
37. Das M, Shehata F, Moria A, Holzer H, Son WY, Tulandi T. Ovarian reserve, response to gonadotropins, and oocyte maturity in women with malignancy. Fertil Steril. 2011;96:122–5.
38. Moria A, Das M, Shehata F, Holzer H, Son W-Y, Tulandi T. Ovarian reserve and oocyte maturity in women with malignancy undergoing in vitro maturation treatment. Fertil Steril. 2011;95:1621–3.
39. Noyes N, Knopman JM, Long K, Coletta JM, Abu-Rustum NR. Fertility considerations in the management of gynecologic malignancies. Gynecol Oncol. 2011;120(3):326–33.
40. Robertson AD, Missmer SA, Ginsburg ES. Embryo yield after in vitro fertilization in women undergoing embryo banking for fertility preservation before chemotherapy. Fertil Steril. 2011;95(2):588–91.
41. Almog B, Eldar I, Hasson Y, et al. Effects of cancer on ovarian response in controlled ovarian stimulation for fertility preservation. Fertil Steril. 2012;98(3):S119.
42. Johnson LN, Dillon KE, Sammel MD, Efymow BL, Mainigi MA, Dokras A, Gracia CR. Response to ovarian stimulation in patients facing gonadotoxic therapy. Reprod Biomed Online. 2013;26:337–44.
43. Levin I, Almog B. Effect of cancer on ovarian function in patients undergoing in vitro fertilization for fertility preservation: a reappraisal. Curr Oncol. 2013;20:e1–3.
44. Nurudeen SK, Douglas NC, Mahany EL, Sauer MV, Choi JM. Fertility preservation decisions among newly diagnosed oncology patients: a single-center experience. Am J Clin Oncol. 2016;39:154–9.
45. Tsampras N, Roberts SA, Gould D, et al. Ovarian response to controlled ovarian stimulation for fertility preservation before oncology treatment: a retrospective cohort of 157 patients. Eur J Cancer Care. 2018;27(2):e12797.
46. Decanter C, Robin G, Mailliez A, Sigala J, Morschhauser F, Ramdane N, Devos P, Dewailly D, Leroy-Martin B, Keller L. Prospective assessment of follicular growth and the oocyte cohort after ovarian stimulation for fertility preservation in 90 cancer patients versus 180 matched controls. Reprod Biomed Online. 2018;36(5):543–51.
47. Visvader JE. Cells of origin in cancer nature. Nature. 2011;469(7330):314–22.
48. Cakmak H, Rosen MP. Ovarian stimulation in cancer patients. Fertil Steril. 2013;99 (6):1476–84.
49. Charlotte S, Marjorie C, Solene D, Christophe S, Nathalie S, Michaël G. Antral follicle responsiveness to FSH, assessed by the follicular output rate (FORT), is altered in Hodgkin's lymphoma when compared with breast cancer candidates for fertility preservation. J Assist Reprod Genet. 2018;35(1):91–7.
50. Kaplan JR, Manuck SB. Ovarian dysfunction, stress, and disease: a primate continuum. ILAR J. 2004;45(2):89–115.
51. Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: a review of the literature. J Assist Reprod Genet. 2013;30(1):11–24.
52. Bornstein SR, Rutkowski H, Vrezas I. Cytokines and steroidogenesis. Mol Cell Endocrinol. 2004;215(1–2):135–41.
53. Paradisi R, Vicenti R, Macciocca M, Seracchioli R, Rossi S, Fabbri R. High cytokine expression and reduced ovarian reserve in patients with Hodgkin lymphoma or non-Hodgkin lymphoma. Fertil Steril. 2016;106:1176–82.
54. Samir M, Glister C, Mattar D, et al. Follicular expression of pro-inflammatory cytokines tumour necrosis factor-α (TNFα), interleukin 6 (IL6) and their receptors in cattle: TNFα, IL6 and macrophages suppress thecal androgen production in vitro. Reproduction. 2017;154:35–49.
55. Fabbri R, Pasquinelli G, Magnani V, Arpinati M, Battaglia C, Paradisi R, Venturoli S. Follicle features in adolescent and young adult women with Hodgkin's disease prior to chemotherapy: a preliminary report. Reprod Biomed Online. 2011;23(6):799–805.

56. Alvarez RM, Ramanathan P. Fertility preservation in female oncology patients: the influence of the type of cancer on ovarian stimulation response. Hum Reprod. 2018;33:2051–9.
57. Carneiro MM. Stem cells and uterine leiomyomas: what is the evidence? J Brasil Reprod Assist. 2016;20(1):33.
58. Kim J, Turan V, Oktay K. Long-term safety of Letrozole and gonadotropin stimulation for fertility preservation in women with breast cancer. J Clin Endocrinol Metab. 2016;101:1364–71.
59. Letourneau JM, Ebbel EE, Katz PP, Oktay KH, McCulloch CE, Ai WZ, Chien AJ, Melisko ME, Cedars MI, Rosen MP. Acute ovarian failure underestimates age-specific reproductive impairment for young women undergoing chemotherapy for cancer. Cancer. 2012;118 (7):1933–9.
60. Stensheim H, Cvancarova M, Møller B, Fosså SD. Pregnancy after adolescent and adult cancer: a population-based matched cohort study. Int J Cancer. 2011;129:1225–36.
61. Quinn MM, Cakmak H, Letourneau JM, Cedars MI, Rosen MP. Response to ovarian stimulation is not impacted by a breast cancer diagnosis. Hum Reprod. 2017; 32(3):568–74.
62. Azim HA, Partridge AH. Biology of breast cancer in young women. Breast Cancer Res. 2014;16:427.
63. Lambertini M, Pinto AC, Ameye L, Jongen L, Del Mastro L, Puglisi F, et al. The prognostic performance of adjuvant! Online and Nottingham prognostic index in young breast cancer patients. Br J Cancer. 2016;115:1471–8.
64. Poggio F, Levaggi A, Lambertini M. Chemotherapy-induced premature ovarian failure and its prevention in premenopausal breast cancer patients. Expert Rev Qual Life Cancer Care. 2016;1:5–7.
65. Derks-Smeets IAP, van Tilborg TC, van Montfoort A, et al. BRCA1 mutation carriers have a lower number of mature oocytes after ovarian stimulation for IVF/PGD. J Assist Reprod Genet. 2017;34:1475–82.
66. Shapira M, Raanani H, Feldman B, Srebnik N, Dereck-Haim S, Manela D, Brenghausen M, Geva-Lerner L, Friedman E, Levi-Lahad E, Goldberg D. BRCA mutation carriers show normal ovarian response in in vitro fertilization cycles. Fertil Steril. 2015;104(5):1162–7.
67. Turan V, Bedoschi G, Emirdar V, Moy F, Oktay K. Ovarian stimulation in patients with cancer: impact of letrozole and BRCA mutations on fertility preservation cycle outcomes. Reprod Sci (Thousand Oaks, CA). 2018;25(1):26–32.
68. Phillips KA, Collins IM, Milne RL, et al. Anti-Müllerian hormone serum concentrations of women with germline BRCA1 or BRCA2 mutations. Hum Reprod. 2016;31(5):1126–32.
69. Lawrenz B, Fehm T, Wolff MV, et al. Reduced pretreatment ovarian reserve in premenopausal female patients with Hodgkin lymphoma or non-Hodgkin-lymphoma—evaluation by using antimüllerian hormone and retrieved oocytes. Fertil Steril. 2012;98(1):141–4.
70. Lekovich J, Lobel ALS, Stewart JD, et al. Female patients with lymphoma demonstrate diminished ovarian reserve even before initiation of chemotherapy when compared with healthy controls and patients with other malignancies. J Assist Reprod Genet. 2016;33 (5):657–62.
71. Sonigo C, Comtet M, Duros S, Sifer C, Sermondade N, Grynberg M. Antral follicle responsiveness to FSH, assessed by the follicular output rate (FORT), is altered in Hodgkin's lymphoma when compared with breast cancer candidates for fertility preservation. J Assist Reprod Genet. 2018;35(1):91–7.
72. van Dorp W, Van Den Heuvel-Eibrink MM, de Vries AC, Pluijm SM, Visser JA, Pieters R, Laven JS. Decreased serum anti-Müllerian hormone levels in girls with newly diagnosed cancer. Hum Reprod. 2014;29(2):337–42.
73. Sklavos MM, Stratton P, Giri N, Alter BP, Savage SA, Pinto LA. Reduced serum levels of anti-Müllerian hormone in females with inherited bone marrow failure syndromes. J Clin Endocrinol Metab. 2015;100(2):E197–203.
74. Donnez J, Dolmans MM. Preservation of fertility in females with haematological malignancy. Br J Haematol. 2011;154(2):175–84.

75. Shapira M, Raanani H, Cohen Y, et al. Fertility preservation in young females with hematological malignancies. Acta Haematol. 2014;132(3–4):400–13.
76. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67(1):7–30.
77. Schover LR, Brey K, Lichtin A, Lipschultz LI, Jeha S. Oncologists' attitudes and practices regarding banking sperm before cancer treatment. J Clin Oncol. 2002;20(7):1890–7.
78. Roeca C, Dovey S, Polotsky AJ. Recommendations for assessing ovarian health and fertility potential in survivors of childhood cancer. Maturitas. 2019;122:57–9.
79. Taylor JF, Ott MA. Fertility preservation after a cancer diagnosis: a systematic review of adolescents', parents', and providers' perspectives, experiences, and preferences. J Pediatr Adolesc Gynecol. 2016;29(6):585–98.
80. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24(18):2917–31.
81. Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, et al. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol. 2009;27(16):2677–85.
82. Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim HA Jr, Peccatori FA, et al. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med. 2016;14:1.
83. Green DM, Sklar CA, Boice JD Jr, Mulvihill JJ, Whitton JA, Stovall M, et al. Ovarian failure and reproductive outcomes after childhood cancer treatment: results from the Childhood Cancer Survivor Study. J Clin Oncol. 2009;27(14):2374–81.
84. Meirow D, Biederman H, Anderson RA, Wallace WH. Toxicity of chemotherapy and radiation on female reproduction. Clin Obstet Gynecol. 2010;53(4):727–39.
85. Van de Loo L, Van den Bergh M, Overbeek A, Van Dijk M, Damen L, et al. Uterine function, pregnancy complications and pregnancy outcomes among female childhood cancer survivors. Fertil Steril. 2019;111:372–80.
86. Eskander RN, Randall LM, Berman ML, Tewari KS, Disaia PJ, Bristow RE. Fertility preserving options in patients with gynecologic malignancies. Am J Obstet Gynecol. 2011;205:103–10.
87. Mancini J, Rey D, Preau M, Malavolti L, Moatti JP. Infertility induced by cancer treatment: inappropriate or no information provided to majority of French survivors of cancer. Fertil Steril. 2008;90(5):1616–25. https://doi.org/10.1016/j.fertnstert.2007.08.064.
88. Peate M, et al. The fertility-related concerns, needs and preferences of younger women with breast cancer: a systematic review. Breast Cancer Res Treat. 2009;116(2):215–23.
89. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364:1405–10.
90. Wallace WHB, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol. 2005;6:209–18.
91. Codacci-Pisanelli G, Del Pup L, Del Grande M, et al. Mechanisms of chemotherapy-induced ovarian damage in breast cancer patients. Crit Rev Oncol Hematol. 2017;113:90–6.
92. Wo J, Viswanathan A. Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients. Int J Radiat Oncol Biol Phys. 2009;5:1304–12.
93. Salama M, Winkler K, Murach KF, Seeber B, Ziehr SC, Wildt L. Female fertility loss and preservation: threats and opportunities. Ann Oncol. 2013;24:598–608.
94. Lawrenz B, Mahajan N, Fatemi HM. The effects of cancer therapy on women's fertility: what do we know now? Future Oncol. 2016;12:1721–9.
95. Alvarez RM, Vazquez-Vicente D. Fertility sparing treatment in borderline ovarian tumours. E Cancer Medical Sci. 2015;9:507.
96. Balachandren N, Davies M. Fertility, ovarian reserve and cancer. Maturitas. 2017;105:64–8.
97. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, Oktay K. American society of clinical oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24:2917–31.

98. Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? Hum Reprod Update. 2012;18:525–35.
99. Behringer K, et al. Secondary amenorrhea after Hodgkin's lymphoma is influenced by age at treatment, stage of disease, chemotherapy regimen, and the use of oral contraceptives during therapy: a report from the German Hodgkin's Lymphoma Study Group. J Clin Oncol. 2005;23 (30):7555.
100. Balcerek M, et al. Suspected infertility after treatment for leukemia and solid tumors in childhood and adolescence. Dtsch Rztebl Int. 2012;109(7):126–31.
101. Bodurka DC, Sun CC. Sexual function after gynecologic cancer. Obstet Gynecol Clin N Am. 2006;33:621–30.
102. Abbott-Anderson K, Kwekkeboom KL. A systematic review of sexual concerns reported by gynecological cancer survivors. Gynecol Oncol. 2012;124:477–89.
103. Pereira N, Hancock K, Cordeiro CN, et al. Comparison of ovarian stimulation response in patients with breast cancer undergoing ovarian stimulation with letrozole and gonadotropins to patients undergoing ovarian stimulation with gonadotropins alone for elective cryopreservation of oocytes? Gynecol Endocrinol. 2016;32(10):823–6.
104. Irtan S, Orbach D, Helfre S, Sarnacki S. Ovarian transposition in prepubescent and adolescent girls with cancer. Lancet Oncol. 2013;14(13):e601–8.
105. Jensen PT, Froeding LP. Pelvic radiotherapy and sexual function in women. Transl Androl Urol. 2015;4(2):186–205.
106. Biedka M, Kuźba-Kryszak T, Nowikiewicz T, Żyromska A. Fertility impairment in radiotherapy. Contemp Oncol (Pozn). 2016;20(3):199–204.
107. Duncan FE, Zelinski M, Gunn AH, et al. Ovarian tissue transport to expand access to fertility preservation: from animals to clinical practice. Reproduction. 2016;152(6):R201.
108. Gao H, Yang BJ, Li N, Feng LM, Shi XY, Zhao WH, et al. Bisphenol A and hormone-associated cancers: current progress and perspectives. Medicine (Baltimore). 2015;94(1):e211.
109. Chieng PU, Huang TS, Chang CC, Chong PN, Tien RD, Su CT. Reduced hypothalamic blood flow after radiation treatment of nasopharyngeal cancer: SPECT studies in 34 patients. Am J Neuroradiol. 1991;12:661–5.
110. Hochberg Z, Kuten A, Hertz P, Tatcher M, Kedar A, Benderly A. The effect of single-dose radiation on cell survival and growth hormone secretion by rat anterior pituitary cells. Radiat Res. 1983;94:508–12.
111. Robinson IC, Fairhall KM, Hendry JH, Shalet SM. Differential radiosensitivity of hypothalamopituitary function in the young adult rat. J Endocrinol. 2001;169:519–26.
112. Marci R, Mallozzi M, Di Benedetto L, et al. Radiations and female fertility. Reprod Biol Endocrinol. 2018;16(1):112.
113. Muñoz M, Santaballa A, Seguí MA, Beato C, de la Cruz S, Espinosa J, et al. SEOM clinical guideline of fertility preservation and reproduction in cancer patients (2016). Clin Transl Oncol. 2016;18(12):1229–36.
114. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod. 2003;18(1):117–21.
115. Damewood MD, Grochow LB. Prospects for fertility after chemotherapy or radiation for neoplastic disease. Fertil Steril. 1986;45(4):443–59.
116. Chemaitilly W, Mertens AC, Mitby P, Whitton J, Stovall M, Yasui Y, et al. Acute ovarian failure in the childhood cancer survivor study. J Clin Endocrinol Metab. 2006;91(5):1723–8.
117. Rodriguez-Wallberg KA, Oktay K. Fertility preservation during cancer treatment: clinical guidelines. Cancer Manag Res. 2014;6:105–17.
118. Green DM, Sklar CA, Boice JD, et al. Ovarian failure and reproductive outcomes after childhood cancer treatment: results from the Childhood Cancer Survivor Study. J Clin Oncol. 2009;27:2374–81.
119. Wallace WHB. Oncofertility and preservation of reproductive capacity in children and young adults. Cancer. 2011;117(Suppl):2301–10.

120. Levy MJ, Stillman RJ. Reproductive potential in survivors of childhood malignancy. Pediatrician. 1991;18(1):61–70.
121. Thibaud E, et al. Ovarian function after bone marrow transplantation during childhood. Bone Marrow Transplant. 1998;21(3):287–90.
122. Beurden MV, Schuster-Uitterhoeve ALJ, Lammes FB. Feasibility of transposition of the ovaries in the surgical and radiotherapeutical treatment of cervical cancer. Eur J Surg Oncol. 1990;16(2):141–6.
123. Mahajan N. Fertility preservation in female cancer patients: an overview. J Hum Reprod Sci. 2015;8(1):3–13.
124. Teh WT, Stern C, Chander S, Hickey M. The impact of uterine radiation on subsequent fertility and pregnancy outcomes. Biomed Res Int. 2014;2014:482968.
125. Stephen MD, Zage PE, Waguespack SG. Gonadotropin-dependent precocious puberty: neoplastic causes and endocrine considerations. Int J Pediatr Endocrinol. 2011;2011(1):184502.
126. Chemaitilly W, Sklar CA. Endocrine complications in longterm survivors of childhood cancers. Endocr Relat Cancer. 2010;17:141–59.
127. Follin C, Erfurth EM. Long-term effect of cranial radiotherapy on pituitary-hypothalamus area in childhood acute lymphoblastic leukemia survivors. Curr Treat Options in Oncol. 2016;17 (9):50.
128. Whitehead E, Shalet SM, Blackledge G, Todd I, Crowther D, Beardwell CG. The effect of combination chemotherapy on ovarian function in women treated for Hodgkin's disease. Cancer. 1983;52(6):988–93.
129. Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update. 2001;7(6):535–43.
130. Duffy C, Allen S. Medical and psychosocial aspects of fertility after cancer. Cancer J. 2009;15 (1):27–33.
131. Marcello MF, Nuciforo G, Romeo R, et al. Structural and ultrastructural study of the ovary in childhood leukemia after successful treatment. Cancer. 1990;66(10):2099–104.
132. Bar-Joseph H, Ben-Aharon I, Tzabari M, Tsarfaty G, Stemmer SM, Shalgi R. In vivo bioimaging as a novel strategy to detect doxorubicin-induced damage to gonadal blood vessels. PLoS One. 2011;6(9):e23492.
133. De Haes H, Olschewski M, Kaufmann M, Schumacher M, Jonat W, Sauerbrei W. Quality of life in goserelin-treated versus cyclophosphamide + methotrexate + fluorouracil-treated premenopausal and perimenopausal patients with node-positive, early breast cancer: the Zoladex Early Breast Cancer Research Association Trialists Group. J Clin Oncol Off J Am Soc Clin Oncol. 2003;21(24):4510–6.
134. Ganz PA, Greendale GA, Petersen L, Kahn B, Bower JE. Breast cancer in younger women: reproductive and late health effects of treatment. J Clin Oncol. 2003;21(22):4184–93.
135. Ghidoni R, Boccardi M, Benussi L, Testa C, Villa A, Pievani M, Gigola L, Sabattoli F, Barbiero L, Frisoni GB, Binetti G. Effects of estrogens on cognition and brain morphology: involvement of the cerebellum. Maturitas. 2006;54:222–8.
136. Nystedt M, Berglund G, Bolund C, Fornander T, Rutqvist LE. Side effects of adjuvant endocrine treatment in premenopausal breast cancer patients: a prospective randomized study. J Clin Oncol. 2003;21(9):1836–44.
137. Tchen N, Juffs HG, Downie FP, Yi QL, Hu H, Chemerynsky I, Clemons M, Crump M, Goss PE, Warr D, Tweedale ME. Cognitive function, fatigue, and menopausal symptoms in women receiving adjuvant chemotherapy for breast cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2003;21(22):4175–83.
138. Algarroba GN, Sanfilippo JS, Valli-Pulaski H. Female fertility preservation in the pediatric and adolescent cancer patient population. Best practice & research. Clin Obstet Gynaecol. 2018;48:147–57.
139. Žulpaitė R, Bumbulienė Ž. Reproductive health of female childhood cancer survivors. Ginekol Pol. 2018;89(5):280–6.

140. Soleimani R, Heytens E, Darzynkiewicz Z, Oktay K. Mechanisms of chemotherapy-induced human ovarian aging: double strand DNA breaks and microvascular compromise. Aging (Albany NY). 2011;3(8):782–93.
141. Codacci-Pisanelli G, Del Pup L, Del Grande M, Peccatori FA. Mechanisms of chemotherapy-induced ovarian damage in breast cancer patients. Crit Rev Oncol Hematol. 2017;113:90–6.
142. Lopes F, Smith R, Anderson RA, et al. Docetaxel induces moderate ovarian toxicity in mice, primarily affecting granulosa cells of early growing follicles. Mol Hum Reprod. 2014;20 (10):948–59.
143. Bedoschi G, Navarro PA, Oktay K. Chemotherapy-induced damage to ovary: mechanisms and clinical impact. Future Oncol. 2016;12(20):2333–44.
144. Apperley J. Issues of imatinib and pregnancy outcome. J Natl Compr Cancer Netw. 2009;7 (10):1050–8.
145. Gosden RG. Fertility preservation: definition, history, and prospect. Semin Reprod Med. 2009;27(6):433–7.
146. Loren AW, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2013;31(19):2500–10.
147. De Vos M, Smitz J, Woodruff TK. Fertility preservation 2 fertility preservation in women with cancer. Lancet. 2014;384(9950):1302–10.
148. Donnez J, Dolmans M-M. Fertility preservation in women. N Engl J Med. 2017;377 (17):1657–65.
149. Bower B, Quinn GP. Fertility preservation in cancer patients: ethical considerations. Adv Exp Med Biol. 2012;732:187–96.
150. Kumar A, et al. Fertility risk discussions in young patients diagnosed with colorectal cancer. Curr Oncol (Toronto, ON). 2012;19(3):155–9.
151. Scanlon M, et al. Patient satisfaction with physician discussions of treatment impact on fertility, menopause and sexual health among pre-menopausal women with cancer. J Cancer. 2012;3:217–25.
152. Maltaris T, et al. The effect of cancer treatment on female fertility and strategies for preserving fertility. Eur J Obstet Gynecol Reprod Biol. 2007;130(2):148–55.
153. Knight LJ, et al. Obstetric management following fertility-sparing radical vaginal trachelectomy for cervical cancer. J Obstet Gynaecol. 2010;30(8):784–9.
154. Leitao MM Jr, Chi DS. Fertility-sparing options for patients with gynecologic malignancies. Oncologist. 2005;10(8):613–22.
155. Wright JD, et al. Fertility preservation in young women with epithelial ovarian cancer. Cancer. 2009;115(18):4118–26.
156. Raju SK, et al. Fertility-sparing surgery for early cervical cancer-approach to less radical surgery. Int J Gynecol Cancer. 2012;22(2):311–7.
157. Rob L, Skapa P, Robova H. Fertility-sparing surgery in patients with cervical cancer. Lancet Oncol. 2011;12(2):192–200.
158. Weinberg LE, Rodriguez G, Hurteau JA. The role of neoadjuvant chemotherapy in treating advanced epithelial ovarian cancer. J Surg Oncol. 2010;101(4):334–43.
159. Chinese Anti-Cancer Association, C.o.B.C.S. Diagnosis and treatment for breast cancer: Chinese anti-Cancer association guideline update. China Oncol. 2015;09:692–754.
160. Oktem O, et al. Ovarian and uterine functions in female survivors of childhood cancers. Oncologist. 2018;23(2):214–24.
161. Terenziani M, et al. Oophoropexy: a relevant role in preservation of ovarian function after pelvic irradiation. Fertil Steril. 2009;91(3):935.e15–6.
162. Blumenfeld Z. How to preserve fertility in young women exposed to chemotherapy? The role of GnRH agonist cotreatment in addition to cryopreservation of embrya, oocytes, or ovaries. Oncologist. 2007;12(9):1044–54.
163. Behringer K, et al. No protection of the ovarian follicle pool with the use of GnRH-analogues or oral contraceptives in young women treated with escalated BEACOPP for advanced-stage

Hodgkin lymphoma. Final results of a phase II trial from the German Hodgkin Study Group. Ann Oncol. 2010;21(10):2052–60.
164. Oktay K, Turkcuoglu I, Rodriguez-Wallberg KA. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. Reprod Biomed Online. 2010;20(6):783–8.
165. Del Mastro 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;306(3):269–76.
166. Munster PN, et al. Randomized trial using gonadotropin-releasing hormone agonist triptorelin for the preservation of ovarian function during (neo)adjuvant chemotherapy for breast cancer. J Clin Oncol. 2012;30(5):533–8.
167. Pantanowitz L, Prefontaine M, Hunt JP. Cholelithiasis of the ovary after laparoscopic cholecystectomy: a case report. J Reprod Med. 2007;52(10):968–70.
168. Kim YB, et al. Progestin alone as primary treatment of endometrial carcinoma in premenopausal women. Report of seven cases and review of the literature. Cancer. 1997;79 (2):320–7.
169. Gunderson CC, et al. Oncologic and reproductive outcomes with progestin therapy in women with endometrial hyperplasia and grade 1 adenocarcinoma: a systematic review. Gynecol Oncol. 2012;125(2):477–82.
170. Kaku T, et al. Conservative therapy for adenocarcinoma and atypical endometrial hyperplasia of the endometrium in young women: central pathologic review and treatment outcome. Cancer Lett. 2001;167(1):39–48.
171. Mitwally MFM, Casper RF. Single-dose administration of an aromatase inhibitor for ovarian stimulation. Fertil Steril. 2005;83(1):229–31.
172. Fatum M, McVeigh E, Child T. The case for aromatase inhibitors use in oncofertility patients. Should aromatase inhibitors be combined with gonadotropin treatment in breast cancer patients undergoing ovarian stimulation for fertility preservation prior to chemotherapy? A debate. Hum Fertil. 2013;16(4):235–40.
173. Donnez J, Dolmans M-M. Fertility preservation in women. Nat Rev Endocrinol. 2013;9 (12):735–49.
174. Kim SS, et al. Recommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. J Assist Reprod Genet. 2012;29(6):465–8.
175. Cobo A, et al. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril. 2016;105(3):755–764.e8.
176. Shi Y, et al. Transfer of fresh versus frozen embryos in ovulatory women. N Engl J Med. 2018;378(2):126–36.
177. Chen Z-J, et al. Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. N Engl J Med. 2016;375(6):523–33.
178. Rienzi L, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update. 2017;23(2):139–55.
179. Metzger ML, et al. Female reproductive health after childhood, adolescent, and young adult cancers: guidelines for the assessment and management of female reproductive complications. J Clin Oncol. 2013;31(9):1239–47.
180. Chian RC, Uzelac PS, Nargund G. In vitro maturation of human immature oocytes for fertility preservation. Fertil Steril. 2013;99(5):1173–81.
181. Green DM, et al. Ovarian failure and reproductive outcomes after childhood cancer treatment: results from the Childhood Cancer Survivor Study. J Clin Oncol. 2009;27(14):2374–81.
182. Wallace WH, et al. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. Lancet Oncol. 2014;15 (10):1129–36.
183. Jadoul P, et al. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. Hum Reprod. 2017;32(5):1046–54.

184. Donnez J, et al. Ovarian tissue cryopreservation and transplantation: a review. Hum Reprod Update. 2006;12(5):519–35.
185. Meirow D, et al. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. Hum Reprod. 2008;23(5):1007–13.
186. Dolmans M-M, et al. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. Blood. 2010;116(16):2908–14.
187. Rosendahl M, et al. Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. Fertil Steril. 2010;94(6):2186–90.
188. Amiot C, et al. Minimal residual disease detection of leukemic cells in ovarian cortex by eight-color flow cytometry. Hum Reprod. 2013;28(8):2157–67.
189. Soares M, et al. Evaluation of a human ovarian follicle isolation technique to obtain disease-free follicle suspensions before safely grafting to cancer patients. Fertil Steril. 2015;104 (3):672–80.
190. Kuleshova L, et al. Birth following vitrification of a small number of human oocytes: case report. Hum Reprod. 1999;14(12):3077–9.
191. Amorim CA, et al. Vitrification as an alternative means of cryopreserving ovarian tissue. Reprod Biomed Online. 2011;23(2):160–86.
192. Sanfilippo S, et al. Vitrification of human ovarian tissue: a practical and relevant alternative to slow freezing. Reprod Biol Endocrinol. 2015;13:67.
193. Shi Q, et al. Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-analysis. Sci Rep. 2017;7(1):8538.
194. Hudson JN, et al. New promising strategies in oncofertility. Expert Rev Qual Life Cancer Care. 2017;2(2):67–78.
195. Luyckx V, et al. A new step toward the artificial ovary: survival and proliferation of isolated murine follicles after autologous transplantation in a fibrin scaffold. Fertil Steril. 2014;101 (4):1149–56.
196. Mouloungui E, et al. A protocol to isolate and qualify purified human preantral follicles in cases of acute leukemia, for future clinical applications. J Ovarian Res. 2018;11(1):4.
197. Truman AM, Tilly JL, Woods DC. Ovarian regeneration: the potential for stem cell contribution in the postnatal ovary to sustained endocrine function. Mol Cell Endocrinol. 2017;445:74–84.
198. Moore HCF, et al. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy. N Engl J Med. 2015;372(10):923–32.
199. Soares M, et al. Eliminating malignant cells from cryopreserved ovarian tissue is possible in leukaemia patients. Br J Haematol. 2017;178(2):231–9.
200. Johnson J, et al. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature. 2004;428(6979):145–50.
201. Hummitzsch K, et al. Stem cells, progenitor cells, and lineage decisions in the ovary. Endocr Rev. 2015;36(1):65–91.
202. Telfer EE, Albertini DF. The quest for human ovarian stem cells. Nat Med. 2012;18(3):353–4.
203. Horan CJ, Williams SA. Oocyte stem cells: fact or fantasy? Reproduction. 2017;154(1): R23–35.
204. Castrillon DH, et al. The human VASA gene is specifically expressed in the germ cell lineage. Proc Natl Acad Sci U S A. 2000;97(17):9585–90.
205. Zou K, et al. Production of offspring from a germline stem cell line derived from neonatal ovaries. Nat Cell Biol. 2009;11(5):631–6.
206. White YAR, et al. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. Nat Med. 2012;18(3):413–21.
207. Hayashi K, et al. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. Science. 2012;338(6109):971–5.
208. Hayashi K, et al. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell. 2011;146(4):519–32.

209. Jain KK. Nanotechnology-based drug delivery for cancer. Technol Cancer Res Treat. 2005;4 (4):407–16.
210. Ahn RW, et al. Nano-encapsulation of arsenic trioxide enhances efficacy against murine lymphoma model while minimizing its impact on ovarian reserve in vitro and in vivo. PLoS One. 2013;8(3):e58491.
211. Waehler R, Russell SJ, Curiel DT. Engineering targeted viral vectors for gene therapy. Nat Rev Genet. 2007;8(8):573–87.
212. Bouard D, Alazard-Dany D, Cosset FL. Viral vectors: from virology to transgene expression. Br J Pharmacol. 2009;157(2):153–65.
213. Davis ME. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. Mol Pharm. 2009;6(3):659–68.
214. Love KT, et al. Lipid-like materials for low-dose, in vivo gene silencing. Proc Natl Acad Sci U S A. 2010;107(5):1864–9.
215. Kaneda Y. Update on non-viral delivery methods for cancer therapy: possibilities of a drug delivery system with anticancer activities beyond delivery as a new therapeutic tool. Expert Opin Drug Deliv. 2010;7(9):1079–93.
216. Vader P, Breakefield XO, Wood MJA. Extracellular vesicles: emerging targets for cancer therapy. Trends Mol Med. 2014;20(7):385–93.
217. Zhen Z, et al. Ferritins as nanoplatforms for imaging and drug delivery. Expert Opin Drug Deliv. 2014;11(12):1913–22.
218. He D, Marles-Wright J. Ferritin family proteins and their use in bionanotechnology. New Biotechnol. 2015;32(6):651–7.
219. Muller LK, Landfester K. Natural liposomes and synthetic polymeric structures for biomedical applications. Biochem Biophys Res Commun. 2015;468(3):411–8.
220. Li L, Zhang L, Knez M. Comparison of two endogenous delivery agents in cancer therapy: Exosomes and ferritin. Pharmacol Res. 2016;110:1–9.
221. Tahover E, Patil YP, Gabizon AA. Emerging delivery systems to reduce doxorubicin cardiotoxicity and improve therapeutic index: focus on liposomes. Anti-Cancer Drugs. 2015;26(3):241–58.