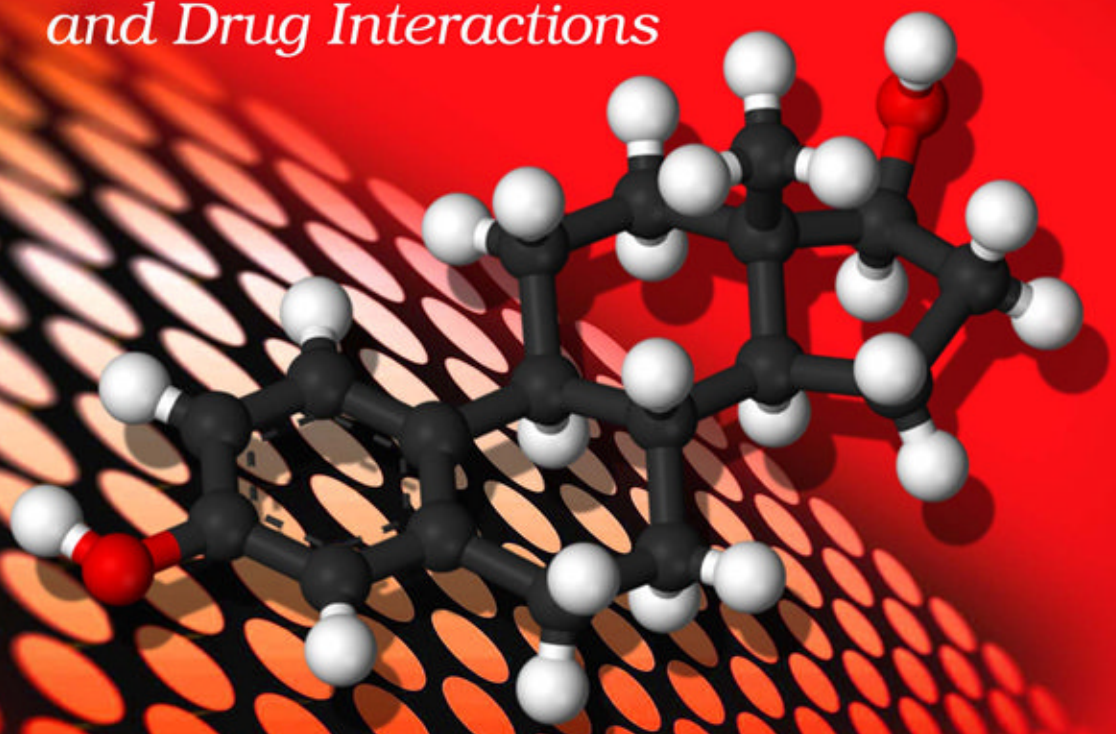
Endocrinology Research and Clinical Developments

# Estradiol

## *Synthesis, Health Effects and Drug Interactions*



Ricco Palmeri
Sal Grimaudo
Editors

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ENDOCRINOLOGY RESEARCH AND CLINICAL DEVELOPMENTS

# ESTRADIOL

# SYNTHESIS, HEALTH EFFECTS AND DRUG INTERACTIONS

# ENDOCRINOLOGY RESEARCH AND CLINICAL DEVELOPMENTS

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# ESTRADIOL

# SYNTHESIS, HEALTH EFFECTS AND DRUG INTERACTIONS

RICCO PALMERI
AND
SAL GRIMAUDO
EDITORS


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# Contents

# Preface

Estradiol is the major steroid hormone which is involved in several organic functions, such as physiological sexual behavior, development and maintenance of male and female reproductive organs, control of visceral secretion, modulation of immune system, and tumorigenesis. In this compilation, the authors discuss the synthesis, health effects and drug interactions of estradiol. Topics include the occurrence of estradiol in environmental waters; estradiol in the central nervous system and its role in neurodegeneration; the effects of estradiol on male and female reproductive tissues and the influence of endocrine disruptors; estradiol and prostate cancer; hydroxyestradiols and methoxyestradiols as endogenous factors associated to physiological and physiopathological conditions; estrogen and functional gastrointestinal disorders; estradiol and memory; estradiol in the environment; use of combined therapy of estrogens with antidepressants; estrogen and nitric oxide as a treatment for diabetes gastroparesis; estradiol synthesis after ovarian tissue cryopreservation and transplantation; estradiol-mediated overmodulation of the neuroendocrine hormone dopamine is central to the pathophysiology of restless leg syndrome during pregnancy; and estrogen modulation on oxidative stress and synaptic plasticity in the dorsal hippocampus.

Chapter 1 - Natural estrogens are a group of steroid hormones that include the main active hormones, 17ß-estradiol (E2), estrone and estriol. Among these compounds, E2 is recognized to be the most active estrogen synthesized in female ovaries.

Environmental problems due to estrogenic compounds are mainly related to aquatic environments. Indeed, numerous studies confirmed the occurrence of these substances at concentrations of toxicological concern (e.g., the feminization of fish in large rivers and toxicological effects on wildlife). Generally, the main sources of estrogens are recognized as treated and untreated municipal and industrial effluents, as well as livestock wastes from agricultural practices, as sewage and manure often used as fertilizers. Moreover the steroid hormones found in the urine of mammals are largely present as inactive conjugates, however the behavior of these forms will deconjugate to rapidly release the free hormones in the environment. As a consequence, large quantities of estrogens are spread in environmental waters, where they may sorb to sediments and persist for relatively long periods.

Recently, the development of new analytical equipment, namely tandem mass spectrometers coupled to LC and GC systems, allowed improvements in the sensitivity, selectivity, and speed of analysis. Such improvements in sensitivity and selectivity could also be accomplished by innovative sample preparation techniques, most of them with the added benefit to be easy to execute, cost effective, and environmental friendly.

Concerning the evaluation of the biological effects of estradiol, several methods have recently been developed: on whole organisms (vitellogenine assay), cells (cell proliferation), yeast estrogen screen, ER CALUX and molecular assays.

In this chapter, various aspects of the estradiol presence in environmental waters are discussed. An overview of the current legislation related to water quality is given. The work then focuses on the health and environmental impacts and evaluation methods. Then, finally, the example case studies illustrate the health effects of estradiol and its environmental impact.

Chapter 2 - Estrogens are one of the main female sex hormones. Within this group, the most active compound with estrogenic action is estradiol. In non-pregnant women, the ovaries are the main place where estradiol is synthesized through a mechanism that depends on pituitary hormones, but there is a well-documented mechanism of estradiol synthesis independent of gonads and pituitary hormones involving the aromatase enzyme, amongst others. Not only has the presence of aromatase been found in some areas of central nervous system, but also changes in its levels after brain damage.

Estradiol is the hormone that is mainly responsible for the development of secondary sexual characteristics in females. In addition to these sexual actions, it can also have effects on the central nervous system in males. Estradiol has been found to modulate different aspects in the brain, not only in normal physiology but also in pathological conditions. It can protect against brain injury, neurodegeneration and cognitive decline, and may act as a "recovery agent*"* in some central nervous system disorders. In recent years, some laboratories have focused on the mechanisms of estradiol used as a neuroprotective agent in some neurodegenerative diseases, although this action is not yet fully understood. Clinical studies have shown that blood levels of estradiol are associated with decreased risk, delayed onset and progression and enhanced recovery from several traumatic or chronic neurological diseases, although there is evidence that estradiol exposure can be deleterious to some neuronal population.

This chapter will focus on the effects of estradiol in the nervous system. The authors will describe some of the recently proposed action mechanisms, summarizing some of the recent progress made on understanding the mechanisms of action of estradiol in the central nervous system including the classical and non-classical receptor-mediated and non-receptor dependent pathways, and bring together some of its putative therapeutic effects for stroke and Alzheimer's disease models. The authors' review focuses on two neurodegenerative diseases with different etiologies and evolution over time. Stroke is a trauma with a fast evolution over time, while Alzheimer's disease is chronic disease that evolves slowly. In both cases, estradiol shows neuroprotective effects in animal models.

Finally, the possibility of using estradiol as a neuroprotector and/or "recovery agent" against some neurodegenerative disorder models is discussed.

Chapter 3 - Estradiol (E2) is the major steroid hormone which is involved in several organic functions, such as physiological sexual behavior, development and maintenance of male and female reproductive organs, control of visceral secretion, modulation of immune system, tumorigenesis, and others. This hormone is essential for the acquisition of secondary sexual characteristics and hypothalamic release of gonadotropin-releasing hormone (GnRH) in mammals. In women, E2 is primarily synthesized by the granulosa cells of the ovary during aromatization of androstenedione (produced in the theca cells) to estrone, and finally to E2. An additional but not significant E2 contribution is derived from liver, adrenal and mammary glands. In both sex, testosterone can be converted into estradiol by aromatization.

In females, E2 has a key role on the functioning of reproductive organs, supporting the lining of the endometrium, cervix, uterine tubes, and vagina. Also, E2 is essential for the growth and maturation of oocytes and to orchestrate folliculogenesis. During menstrual cycle, E2 triggers the hypothalamic-pituitary events via feedback system to induce ovulation, besides participating with progesterone in the preparation of endometrium for blastocyst implantation. In males, the effect of E2 seems to be more complex. E2 is produced by the activity of aromatase mainly in leydig cells of the testis. Current researches have provided evidences that E2 is not only responsible for differentiation and function of the testes, but it may also exert actions in the epididymis, efferent ductules, prostate, seminal vesicles, and even the penis. Furthermore, excessive estrogen exposure has been postulated to be the main cause of low sperm counts which can lead to subfertility or infertility-related disorders.

E2 binds to estrogen receptors (namely ERα and ERβ subunits) at nuclear level and stimulates selective genes transcription in a tissue and cell-specific manner. The effective activation of the ERs by E2 is strongly associated with an estrogen responsive element (ERE) in the promoter region of the target genes to control its activation and/or repression. There is a variety of medications that preferentially act on one of these ER to exert such effects. Notably, endocrine-disrupting chemicals (EDCs) are synthetic or natural compounds found in the environment which interfere with hormone production and physiological functions of the reproductive organs. The most EDCs affecting reproductive function include synthetic agents used as polychlorinated biphenyls, polybrominated biphenyls, methoxychlor (MXC), phthalates, dichlorodiphenyltrichloroethane (DDT), diethylstilbestrol (DES), and other related compounds. The naturally occurring agents with estrogenic activity are originated from phytoestrogens (e.g., genistein and coumestrol). This chapter will give attention to the main actions of E2 on both male and female reproductive organs and discuss on the specific toxicity of EDCs to the reproductive function.

Chapter 4 - Although the most impressive characteristic of prostate cancer is its androgen dependence, several studies support that 17β-estradiol ($E_2$) also plays an important role in onset and progression of prostate cancer. Regarding the effects of $E_2$ in prostate carcinogenesis, the authors can highlight the carcinogenic properties of several products derived from $E_2$ metabolism, which play an important role on cell malignant transformation. Several polymorphisms in estrogen-related genes such as estrogen receptors (ERs) and enzymes involved in $E_2$ biosynthesis and metabolism have been described to favour carcinogenesis. Also, both isoforms of ERs (ERα and ERβ), which act on cells in order to maintain the normal physiology of prostate gland, are differentially expressed between neoplastic and non-neoplastic prostate cells. Therefore, this deregulation may conduct to alterations on normal gene expression in prostate cells, which may favour the progression and migration of cancer cells. On the other hand, other studies have been pointing the anti-carcinogenic activity of $E_2$ in prostate. These contradictory effects are based on the role of ERβ, which has been shown to exhibit anti-proliferative and anti-oxidant functions. Taking into account the scientific evidences that relate $E_2$ with prostate cancer, several therapeutic approaches based on ERs are being explored. This chapter summarizes the main knowledge on how $E_2$ may contribute to the pathophysiology of prostate gland.

In developed countries, prostate cancer (PCa) is the most frequently diagnosed malignancy in men. The aetiology of this disease is complex and remains unclear. Risk factors for a high morbidity of PCa can be classified as endogenous (family history, hormones, race, aging and oxidative stress) or exogenous (dietary factors, physical inactivity,

obesity, environmental factors, occupation, smoking, sexual activity, vasectomy). However, a positive family history is the strongest epidemiological risk factor for PCa.

The relationship between hormones and the pathogenesis of PCa has been extensively studied. PCa is generally considered a paradigm of androgen-dependent tumour. However, estrogens role appears to be equally important in both normal and malignant prostate. Recent epidemiologic and experimental data have clearly pinpointed the key roles of estrogens in PCa development and progression. PCa risk in adulthood could be determined by exposure to estrogens during embryonic, perinatal/neonatal, or peripubertal development, a phenomenon referred to as ‘‘estrogen imprinting’’. Estrogens action should be considered both at a systemic endocrine level, because these steroid hormones are able to act through the pituitary gland to indirectly lower androgens, and locally within the prostate tissue . Salonia and collaborators demonstrated for the first time that the circulating 17β-estradiol ($E_2$, the most potent subtype of estrogens) levels are associated with a greater incidence of high grade PCa. Patients with high $E_2$ levels have an increased risk to develop PCa. Another study also demonstrated that $E_2$ exposure could neoplastically transform the rat prostatic epithelial cells *in vitro*. These results support the idea that estrogens have long been implicated in the prostate carcinogenesis. In addition, high estrogens levels and low testosterone levels induce the development of inflammation upon aging and the onset of premalignant lesions.

Understanding how estrogens are synthesized and act on prostate cells, will indubitably contribute to better understand the molecular mechanisms underlying PCa onset and progression, as well as to delineate novel strategies in diagnosis and treatment of PCa.

Chapter 5 - Estradiol ($E_2$) is a steroidal hormone generated by the conversion of testosterone via the p450 aromatase enzymatic complex. The $E_2$ physiological actions are mainly mediated by its interaction with intracellular receptors known as estrogen receptors (ERs). The $E_2$-ERs complex is able to alter the gene expression in its target cells binding to specific sequences in the DNA. Besides, estrogens can also activate several intracellular signal transduction cascades (e.g., cAMP-PKA, IP3-$Ca^{2+}$) by non-genomic mechanisms.

Following to exert its biological effects in their target tissues, $E_2$ must be inactivated and eliminated by the body through its conversion to soluble compounds with a insignificant or very low estrogenic activity. These reactions are accomplished by several enzymatic processes that involve reactions of oxidations and conjugations. The enzymatic modifications that a molecule of $E_2$ undergoes to be eliminated include sulfonations, O-methylations, hydroxylations and glucurodinations. Even though the conversion of $E_2$ to inactive or less active metabolites occurs mainly in the liver, it has been reported that some peripheral tissues, including breast, uterus, placenta and brain, express the enzymes required to inactive $E_2$.

One of the most studied enzymatic pathways that inactive estradiol in peripheral tissues consists in a C-2 hydroxylation, a reaction catalyzed by the enzyme cytochrome p450, isoform 1A1 (CYP1A1), that generates a molecule of 2-hydroxyestradiol ($2OHE_2$) and the C-4 hydroxylation, a reaction catalyzed by the enzyme CYP1B1 that generates 4-hydroxyestradiol ($4OHE_2$). Then, the hydroxyl group previously added is replaced by a methyl group through a conjugation reaction catalyzed by the enzyme Catechol-O-Methyltransferase (COMT), which originates a molecule of 2- methoxyestradiol ($2ME_2$) from $2OHE_2$ and 4-methoxyestradiol ($4ME_2$) from $4OHE_2$.

Recently, it has been demonstrated that hydroxyestradiols and methoxyestradiols are not inactive molecules since several reports have shown that these estradiol metabolites may exert physiological actions in different organs and tissues, while an unbalanced estradiol

metabolization to hydroxyestradiols and methoxyestradiols could be the responsible factor of several diseases including cancer and preeclampsia. In this chapter the authors will review the available literature concerning to the physiological effects that hydroxyestradiols and methoxyestradiols exert in several organs and how an altered production of hydroxyestradiols or methoxyestradiols could have deleterious effects on several biological functions. They will specially discuss the possible physiological and physiopathological effects of $2ME_2$ in female reproductive tissues, where this estradiol metabolite is able to alter the ovum transport and change the gene expression profile. Particularly, the authors will describe a group of $2ME_2$-induced genes in the mouse uterus that could be useful as biomarkers to elucidate the role of $2ME_2$ in the female reproductive tract.

Chapter 6 - This chapter will review: 1) the pathophysiological mechanisms of functional gastrointestinal disorders (FGIDs); 2) the effects of estrogen on motor dysfunction, visceral pain, gastrointestinal (GI) symptoms and some FGIDs such as gastroesophageal reflux disease (GERD), functional dyspepsia (FD), gastroparesis, irritable bowel syndrome (IBS) and constipation.

Chapter 7 - The role of estradiol as a sex hormone is well-established. However, estradiol also exerts actions in the central nervous system that extend beyond basic sexual and reproductive functions. In this respect, estradiol exerts pronounced effects on the morphology and function of brain regions not typically considered for their contribution to reproductive function. As such, circulating levels of estradiol have been shown to produce marked effects on both reproductive and non-reproductive behaviors. The well-documented effects of estradiol on dendritic spine density in hippocampal pyramidal cells, an area critical to learning and memory, indicate an important role for estradiol in memory function. These "non-reproductive" behavioural effects remain unclear and the precise actions of estradiol on memory function are yet to be fully elucidated. The effects of estradiol on the brain throughout the lifespan are of particular interest due to the therapeutic implications which may be derived. While this is of particular interest during aging, it is also important to examine the effects of estradiol on the young adult and adolescent brain to determine the functional implications of estradiol's effects. A review of the pertinent literature concerning estradiol's morphological consequences in the brain, in particular, the hippocampus, and effects on memory function will be examined.

Chapter 8 - In last decades, increases in the occurrence of certain abnormalities in development of wildlife species brought world-wide attention to scientific community about environmental contaminants. Estradiol is a steroidal natural hormone produced by humans and animals that is excreted in urine and feces, reaching the natural environment mainly through discharge from wastewater treatment plants (WWTP). The environmental occurrence of estradiol leads to increase the frequency of appearance of reproductive disorders and cancer in wildlife and probably in humans, being nanogram-per-liter levels of this compound able to produce endocrine disruption in living organisms. Reported concentrations of estradiol in environmental matrices are usually very low and the analytical difficulties associated with the determination of this estrogen in environment have been overcome by using preconcentration and clean up steps followed by highly sensitive analytical methods and the number of analytical methods currently available for the determination of estradiol in environmental matrices is still limited.

The purpose of this chapter is to provide a general overview on estradiol occurrence and analysis in environmental matrices.

Chapter 9 - Depression is an important health problem, particularly in women around perimenopause. Controversial results exist in the efficacy of the combination of estrogens with antidepressants for depression treatment. Indeed, the use of estrogens as replacement therapy or as co-adjuvant with antidepressants has been subject of intense debate due to the possibility that estrogens may induce carcinogenic effects. However, recent reports indicate that the age of intervention -in relation with the menopause onset- is a crucial factor to explain the failure or success of the combined treatment with estrogens and antidepressants.

Other feature that contributes to the controversy is the type of compounds. Thus, the estrogenic compounds -with divergent neuropharmacological properties- differentially interact with the dissimilar kind of antidepressants: selective serotonin reuptake inhibitors, mixed reuptake inhibitors, tricyclics, atypical and monoamine-oxidase inhibitors.

The present review analyzes these two factors: age of treatment in relation to loss of ovarian function and type of estrogen/antidepressant. Thus, in the first part is presented a brief panorama of clinical and preclinical data of combining estrogenic compounds (17-β estradiol, $E_2$ and 17-α ethinyl-estradiol, $EE_2$) and antidepressants that the authors have used in basic research studies: two selective serotonin reuptake inhibitors (fluoxetine and citalopram), a mixed serotonin-noradrenaline reuptake inhibitor (venlafaxine) and a tricyclic compound preferentially with noradrenergic actions (desipramine).

Secondly, the antidepressant effects of the selective serotonin reuptake inhibitors are analyzed in relation to the subject's age and the time of loss of ovarian activity (menopause or ovariectomy) in clinical and preclinical studies. Finally, the participation of different monoaminergic and estrogen receptors as mediators of the synergism observed between estrogens and antidepressants is discussed.

Chapter 10 - Diabetic gastroparesis, defined as delayed gastric emptying, is one of the most debilitating complications of both type 1 and type 2 diabetes. Normal gastric motility/emptying requires an integrated, coordinated interplay between sympathetic, parasympathetic, and intrinsic-gut (enteric) nervous systems, interstitial cells of Cajal, and gastrointestinal smooth muscle cells. Any disturbance in their interactions has the potential to alter gastric function, and ultimately affects gastric emptying. Multiple contributing factors, including depletion of interstitial cells of Cajal, oxidative stress, excitatory and inhibitory neuropeptides, estrogen, and the nitric oxide regulator of neuronal nitric oxide synthase activity (nNOSα), have been linked to the development of gastroparesis.

Gastroparesis is far more common in women (60-70%), suggesting a possible estrogen role in gastric emptying dysfunction. Several animal and human studies have demonstrated that estradiol-17β causes delayed gastric emptying. In one study, estrogen administration in overiectomized rats, as well as postmenopausal women receieving estrogen replacement therapy, resulted in delayed gastric emptying.

Nitric oxide is a major neurotransmitter in the gut and a key physiological mediator of non-adrenergic and non-cholinergic relaxation of gastrointestinal smooth muscle cells. There is a myriad of evidence suggesting that gastroparesis is associated with changes in gastric neuronal nitric oxide synthase (nNOSα) expression and its activation state, which directly regulates the bioavailability of nitric oxide. Taken together, these evidences suggest the estrogen's role in normal gastric emptying may be via a nitric oxide regulatory pathway. The authors recently developed an animal model of diabetic gastroparesis by injecting male and female rats with streptozotocin. Male diabetic rats without gastroparesis were subsequently injected with estrogen for 3 weeks and then evaluated for the development of gastroparesis.

Although male diabetic gastroparetic (either streptozotocin- or estrogen-induced) rats exhibited similarity in disease pathology to that of females, the molecular mechanisms of development were different. The authors' results indicated that slow gastric emptying in both male diabetic groups was not associated with the level of expression and dimerization of nNOSα in gastric tissues. In contrast, females with diabetic gastroparesis demonstrated significantly impaired levels and dimerization of nNOSα in the antrum and pylorus. These results open new considerations for a potential role that estrogen may play in normal gastric emptying via a nitric oxide regulatory pathway.

Given the importance of nitric oxide in health and disease, studies on estrogen mediated regulation of nitric oxide will offer a better understanding of gastroparesis and potential development of new therapeutic interventions. In this article, the authors provide a synopsis of studies linking estrogen and nitric oxide to the development of gastroparesis. They discuss basic knowledge and their current understanding of mechanisms for gastroparesis development. Finally, perspectives on future directions of research on gastroparesis are discussed.

Chapter 11 - Fertility preservation is an effort to help cancer patients retain their fertility and an emerging discipline that now plays a central role in the care of reproductive women with cancer. An increasingly larger number of women are surviving with cancer because of improvement in diagnostic and therapeutic strategies. As a result, quality-of-life issues, including issues involving fertility preservation, have gained significant importance in cancer care. Breast cancer is the most common cancer in reproductive women in the US. Most women with breast cancer require the adjuvant chemotherapy including cyclophosphamide.

Cytotoxic treatment such as chemotherapy and/or radiotherapy can cause severe gonadal damages resulting in premature ovarian failure and infertility in female. Adjuvant chemotherapy particularly with alkylating agents such as cyclophosphamide is gonadotoxic and induces premature ovarian failure. The American Society of Clinical Oncology issued practice guideline update for fertility preservation options in cancer patients. Several well established methods for fertility preservation were introduced such as embryo and oocyte cryopreservation, gonadal shielding during radiotherapy, trachelectomy, and ovarian transposition. For fertility preservation, ovarian stimulation with gonadotropins for embryo or oocyte cryopreservation results in excessive levels of estrogen production. To reduce the estrogen exposure during ovarian stimulation in hormone dependent cancer, a novel protocol using letrozole (aromatase inhibitor) and gonadotropins was developed.

Ovarian tissue cryopreservation and transplantation is a main option to preserve their fertility in cancer patients who need cancer treatments without delay or do not want to undergo ovarian stimulation. For prepubertal girls diagnosed with cancer, ovarian tissue freezing is the only option for fertility preservation. Based on the site of transplantation of cryopreserved ovarian tissue, transplantation can be divided into two different types as orthotopic and heterotopic transplantation.

In a recent review of 60 cases of frozen-thawed ovarian transplantation, all ovarian tissue were frozen with the slow-freezing method and the restoration of ovarian activity was observed in at least 92.9%. In all cases, it took 3.5–6.5 months after transplantation before a rise in E2 and a decrease in FSH were detected (mean 4.5 months). To date, a total of 24 live births and 4 ongoing pregnancies have been reported worldwide from ovarian tissue cryopreservation and transplantation. Based on their review, the authors believe that ovarian

cryopreservation and transplantation before cancer treatments is an effective option to preserve their fertility and to restore gonadal endocrine function including estradiol synthesis.

Chapter 12 - A large corpus of evidence points to an imbalance between the classical thyroid gland hormone (TH) and the neuroendocrine hormone dopamine (DA,[IMBTH/DA]) as the main derangement underpinning the pathophysiology of restless legs syndrome (RLS). DA is released by the hypothalamus into the portal blood circulation of the pituitary stalk, where it modulates the release of thyrotropin by the pituitary: DA inhibits the synthesis of the two component subunits of thyrotropin, alpha and beta. Furthermore, the dopaminergic system enhances the activity of the cytochrome P450 superfamily of enzymes via mesolimbic and tuberoinfundibular pathways. As nearly 20% of thyroid hormone is metabolized in these pathways, when DA activity increases, TH activity decreases. TH is essential for somatosensory system physiology, including the sensitivity of somatosensory receptors deep in the legs. When the thresholds of these somatic receptors are diminished, or when their signaling to the sensory cortex is too strongly transmitted, RLS may ensue. One action of TH is to positively influence the sensitivity of the receptors and the strength of their signal transmission to the sensory cortex. DA modulates this TH activity, and when DA is diminished, TH is increased, which may result in IMBTH/DA and RLS. RLS is more common in pregnant than in non-pregnant women, and during pregnancy, TH activity increases to meet the augmented metabolic necessities of pregnancy. Estradiol during pregnancy increases exponentially, and one of the effects of estradiol is the inhibition of DA release in the pituitary stalk. Furthermore, estradiol also increases thyrotropin release by increasing the sensitivity of thyrotrophic cells in the pituitary to thyrotropin-releasing hormone from the hypothalamus. It is known that estradiol levels in RLS pregnant women are higher than those in non-RLS pregnant women. In this chapter, the authors present evidence that suggests that excessive estradiol-mediated modulation of DA is central to the pathophysiology of RLS during pregnancy.

Chapter 13 - Estrogens are important for antioxidant mechanisms, maintenance of synaptic plasticity, and modulation of zinc content in the hippocampus of young and senile animals. The female brain is influenced by multiple factors including the decrease in estrogen levels, with that commonly accompanies stress and aging. However, plasticity in the hippocampus in pre- and postsynaptic sites, such as the mossy fiber system and dendritic spines, responds to the neuroprotective and antioxidant actions of hormone supplementation. Furthermore, in hippocampus, the ovariectomy produces spine pruning in pyramidal cells of CA1, a reduction of the mossy fiber area in CA3, a decrease of the zinc level in the whole hippocampus and an increase in oxidative stress. Following supplementation with a minimal dose of 17β-estradiol, these effects are reversed and these parameters have values similar to those observed during the proestrus stage of the estrous cycle, which is associated with a better performance in learning and memory in female rats. These studies confirm the roles of estrogen in protecting the hippocampus before oxidative stress and maintaining its synaptic plasticity.

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*Chapter 1*

# Occurrence of Estradiol in Environmental Waters

***Valentina F. Domingues[1], Virgínia C. Fernandes[1], Luísa Correia-Sá[1] and Catarina Mansilha[2,3]***
[1]REQUIMTE, Instituto Superior de Engenharia,
Instituto Politécnico do Porto, Porto Portugal
[2]Instituto Nacional de Saúde Dr. Ricardo Jorgem Porto, Portugal
[3]REQUIMTE, Universidade do Porto, Porto, Portugal

## Abstract

Natural estrogens are a group of steroid hormones that include the main active hormones, 17ß-estradiol (E2), estrone and estriol. Among these compounds, E2 is recognized to be the most active estrogen synthesized in female ovaries.

Environmental problems due to estrogenic compounds are mainly related to aquatic environments. Indeed, numerous studies confirmed the occurrence of these substances at concentrations of toxicological concern (e.g., the feminization of fish in large rivers and toxicological effects on wildlife). Generally, the main sources of estrogens are recognized as treated and untreated municipal and industrial effluents, as well as livestock wastes from agricultural practices, as sewage and manure often used as fertilizers. Moreover the steroid hormones found in the urine of mammals are largely present as inactive conjugates, however the behavior of these forms will deconjugate to rapidly release the free hormones in the environment. As a consequence, large quantities of estrogens are spread in environmental waters, where they may sorb to sediments and persist for relatively long periods.

Recently, the development of new analytical equipment, namely tandem mass spectrometers coupled to LC and GC systems, allowed improvements in the sensitivity, selectivity, and speed of analysis. Such improvements in sensitivity and selectivity could also be accomplished by innovative sample preparation techniques, most of them with the added benefit to be easy to execute, cost effective, and environmental friendly.

Concerning the evaluation of the biological effects of estradiol, several methods have recently been developed: on whole organisms (vitellogenine assay), cells (cell proliferation), yeast estrogen screen, ER CALUX and molecular assays.

In this chapter, various aspects of the estradiol presence in environmental waters are discussed. An overview of the current legislation related to water quality is given. The work then focuses on the health and environmental impacts and evaluation methods. Then, finally, the example case studies illustrate the health effects of estradiol and its environmental impact.

# 1. Introduction

The endocrine system is composed of different glands that control hormone metabolism. Hormones are fundamental for the regulation of a variety of biological functions including growth, metabolism, tissue function and differentiation, sexual development and behavior, and also the development of the immune system. Most hormones bind to specific membrane receptors on target cells, activating a cascade of biochemical reactions that eventually lead to the intended effect (e.g., development of a certain tissue type). However some lipophilic hormones (such as steroid and thyroid hormones) bind directly to intracellular receptors. This receptor-hormone complex interacts with transcription-control sequences of the DNA, thus modulating RNA and protein synthesis of specific genes(Leusch et al., 2006).

Natural steroids are secreted by the adrenal cortex, testis, ovary and placenta in human and animal, and include progestogens, glucocorticoids, mineralocorticoids, androgens and estrogens. Estrogens (estradiol (E2), estrone (E1) and estriol (E3)) are predominantly female hormones, which are important for maintaining the health of the reproductive tissues, breasts, skin and brain (Ying et al., 2002; Silva et al., 2012).

Steroid hormones are essential for reproduction, stress management, salt and glucose balances, as well as several other physiological processes. Due to the relatively simple chemical structure and lipophilic nature of steroids, their regulatory pathways can easily be modified by pharmacological, environmental, and/or dietary agents. Because of this, steroids or steroid-mimicking compounds are applied in many fields, making the identification of the endocrine activity of these compounds important (Sonneveld et al., 2006).

Both natural hormones (i.e. E1, 17α-estradiol and 17β-estradiol) and synthetic hormones (EE2) have the potential to behave like endocrine disrupting compounds (EDC) in the environment.

All humans, as well as animals, excrete natural and synthetic hormones in different amounts, depending on age, state of health, diet, or pregnancy (Ying et al., 2002; Silva et al., 2012). On average a menstruating female excretes 8 µg/day and a pregnant woman 600 µg/day, males also excrete E2 in a daily average of 3.9 µg/day (Johnson et al., 2000). Many of these hormones are peptides and are rapidly destroyed. However, the steroid hormones are chemically very stable and are excreted in the free form or as conjugates, which readily biotransform to the free form (Adlercreutz et al., 1987; Shore and Shemesh, 2003). One of the steroids of major concern is the E2, since it exerts physiological effects at lower concentrations than other steroids and can be found in the environment in concentrations above its lowest observable effect level (LOEL) for fish and plants (10 ng/L) (Panter et al., 1998; Irwin et al., 2001; Shore and Shemesh, 2003; Hamid and Eskicioglu, 2012).

Hormone steroids excreted by humans and animals enter the environment through the discharge of domestic sewage effluents and disposal of animal waste. Natural sources include

human and animal hormones and phyto- and mycoestrogens that are intentionally or accidentally included in food and feed ingredients (Goksoyr, 2006).

These compounds could affect wildlife and human health by disrupting their normal endocrine systems. Hormone steroids have been detected in wastewater effluents and surface water as well as ground water at various levels. The behavior and fate of these hormone steroids in the environment depend on their physicochemical properties and environmental media (Yin et al., 2002).

The concern with water quality in terms of their estrogen and xenoestrogen content is an important issue when the contamination of drinking water supplies is considered. Several authors reported different analytical procedures and showed the presence of different hormones in water samples. Almost all processes operating in conventional water treatment plants (WTP) were not designed to remove such compounds leading to the occurrence of emerging contaminants in drinking, finished and/or tap waters (Jardim et al., 2012).

## 2. Impact of Estradiol in the Environment and Health

A growing body of scientific research indicates that some substances present in the environment may interfere with the normal function of the endocrine system of wildlife and humans (Silva et al., 2012). Since the magnitude of risks related to the presence of several EDCs (Endocrine Disrupting Chemicals), including the steroid hormones, in the environment is difficult to predict, large concern exists about these substances (Silva et al., 2012). When present in environment above a certain concentration (threshold limit value), these compounds can cause adverse health effects on wildlife (Hamid and Eskicioglu, 2012).

Among these different classes of EDCs, human and animal waste born hormones, often known as endogenous steroidal hormones, have been characterized by very high estrogenic potency (Hamid and Eskicioglu, 2012). Hormones act at extremely low levels (parts per trillion); therefore, in theory, even exposures to low levels of hormonally active agents may be of concern, particularly during sensitive periods of fetal development. Furthermore, endocrine-mediated effects may be subtle and manifest primarily in populations rather than in individuals (Solomon and Schettler, 2000). Furthermore, in the environment, chemical interactions may have profound consequences, since organisms are likely to be exposed to complex chemical mixtures of environmental pollutants (Lyssimachou and Arukwe, 2007). These complex chemical interactions have only recently become the focus of systematic investigation (Lyssimachou and Arukwe, 2007).

The presence of estrogenic compounds in the environment has become a concern because they may interfere with the reproduction of man, livestock and wildlife (Yin et al., 2002).

Natural hormones display the highest affinity for binding to nuclear estrogen receptors (ERs) and present the greatest estrogenic potency. Due to the strong estrogen affinity for ERs, the presence of low estrogen concentrations in the environment may produce endocrine disruption in a wide range of wildlife populations (Combalbert and Hernandez-Raquet, 2010).

It has now been clearly established that a number of chemical compounds and natural substances present in the aquatic environment are able to disturb the normal physiology and endocrinology of organisms (Brion et al., 2004). The natural steroid estrogens, namely the E2

have been measured in industrial and municipal sewage treatments works (STW) effluents and these discharges represent the main source of synthetic and natural estrogens into the aquatic system/environment. Nonetheless, the surface runoff is another possible source of estrogenic contamination (Brion et al., 2004). The E2 has also been detected in sewage treatment effluents. The origin of the natural steroid estrogens is predominantly from humans, and another source is from the use of conjugated estrogens for the treatment of cancer, osteoporosis, the menopause and hypogonadism. More diffuse sources come from agricultural practices (Brion et al., 2004).

In European countries, the concentrations of E2 in effluents from sewage treatment works range from low nanograms per liter up to hundreds of nanograms per liter. However, surface waters adjacent to discharges from STW effluents can receive considerable quantities of steroid estrogens (Brion et al., 2004).

E2 that is released by humans and livestock in the environment, can be considered one of the most potent endocrine disrupters even at nanogram per liter levels (Khanal et al., 2006). E2 is the main compound responsible for the estrogenic activity in sewage treatment works effluents, and given the reported concentrations in surface waters, and its estrogenic potency, E2 is now considered as an important contaminant of the aquatic systems (Brion et al., 2004). But this effect is not limited to aquatic organisms and therefore, nowadays the concern about the effect of E2, present in the environment, on human health is rising.

Indeed, estrogen hormones found in the environment appear to have physiological effects at very low concentrations, with the most supportive data being related to the effects on development, sexual differentiation, vitellogenin (VTG) production (the precursor of egg yolk protein), and reproduction of diverse fish species (Combalbert and Hernandez-Raquet, 2010) (table 1). These studies have been conducted in a variety of fish species from a number of different families but Medaka fish is the best represented. The results revealed that the major health effects (table 1) associated with exposure of different fish species to E2 included altered sexual development, presence of intersex species, changed mating behavior, decreased production and egg fertility, VTG induction, reduced male gonadosomatic index (GSI), etc.

It was showed that concentrations of E2 found in the environment could have disruptive effects on key steroidogenic enzyme pathways that control sexual development in fish (Halm et al., 2002).

The life stage of fish subjected to E2 exposure seems to have an impact in health outcome, namely in the Rare minnows. It was observed that the juvenile stage could be a critical period for the gonad development (Liao et al., 2009).

Recently Lee, Kanget et al.(Lee et al., 2012) also access the exposure effect on Medaka fish development, observing that the exposure to several EDCs including E2 significantly decreased the fluorescent intensity of the GnRH3 (Gonadotropin-releasing hormone) neurons, postponed the eye development, and retarded the growth of the embryos. Roepke et al. (Roepke et al., 2005) also mentioned that E2 and estrogenic EDCs all caused development toxicity in sea urchins through a TAM-sensitive (tamoxifen-sensitive manner) mechanism. Diotel, Nicolas et al. (Diotel et al., 2013) alos reported that E2 had an inhibitory effect on adult neurogenesis in gonad-intact adult zebrafish.

Other health effect due to E2 exposure was reported (Lerner et al., 2007) in a study conducted in an Atlantic salmon. In this experiment authors evaluated the effect of a treatment with 4-Nonylphenol (NP-L) or E2 in larvae. The authors observed a decreased gill sodium-potassium-activated adenosine triphosphatase (Na+,K+-ATPase) activity and