Chapter 1

# Gonadal Sex Steroids: Production, Action and Interactions in Mammals

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Additional information is available at the end of the chapter

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## 1. Introduction

There are five major classes of steroid hormones: testosterone (androgen), estradiol (estrogen), progesterone (progestin), cortisol/corticosterone (glucocorticoid), and aldosterone (mineralocorticoids). Testosterone and its more potent metabolite dihydrotestosterone (DHT), progesterone and estradiol are classified as sex-steroids, whereas cortisol/corticosterone and aldosterone are collectively referred to as corticosteroids.

Sex steroids are crucial hormones for the proper development and function of the body; they regulate sexual differentiation, the secondary sex characteristics, and sexual behavior patterns. Sex hormones production is sexually dimorphic, and involves differences not only in hormonal action but also in regulation and temporal patterns of production. Gonadal sex steroids effects are mediated by slow genomic mechanisms through nuclear receptors as well as by fast nongenomic mechanisms through membrane-associated receptors and signaling cascades. The term *sex steroids* is nearly always synonymous with *sex hormones* (Wikipedia).

Steroid hormones in mammals regulate diverse physiological functions such as reproduction, mainly by the hypothalamic-pituitary-gonadal axis, blood salt balance, maintenance of secondary sexual characteristics, response to stress, neuronal function and various metabolic processes(fat, muscle, bone mass). The panoply of effects, regulations and interactions of gonadal sex steroids in mammals is in part discussed in this chapter.

## 2. Production of gonadal steroids

Cholesterol is found only in animals; it is not found in plants although they can produce phytoestrogens from cholesterol-like compounds called phytosterols.

Because cholesterol cannot be dissolved in the blood, it must be carried through the body on a "carrier" known as a lipoprotein. A lipoprotein is cholesterol covered by protein. There are two types of liproproteins-LDL (low density lipoprotein) and HDL (high density lipoprotein). All steroid hormones are synthesized from cholesterol through a common precursor steroid, pregnenolone, which is formed by the enzymatic cleavage of a 6-carbon side-chain of the 27- carbon cholesterol molecule, a reaction catalyzed by the cytochrome P450 side-chain cleavage enzyme (P450scc, CYP11A1) at the mitochondria level (Figure 1a). The ovarian granulosa cells mainly secrete progesterone (P4) and estradiol (E2); ovarian theca cells predominantly synthesize androgens,and ovarian luteal cells secrete P4 (and its metabolite 20$\alpha$-hydroxyprogesterone (Hu et al., 2010). Progesterone is also synthesized by the corpus luteum and by the placenta in many species as it will be mentioned later. Testicular Leydig cells are the site of testosterone (T) production. The brain also synthesizes steroids *de novo* from cholesterol through mechanisms that are at least partly independent of peripheral steroidogenic cells. Such *de novo* synthesized brain steroids are commonly referred to as neurosteroids. In mammals, the adrenal or suprarrenal glands are endocrine glands that produce at the outer adrenal cortex androgens such as androstenedione.

All these steroidogenic tissues and cells have the potential to obtain cholesterol for steroid synthesis from at least four potential sources: a) cholesterol synthesized *de novo* from acetate;b) cholesterol obtained from plasma low-density lipoprotein (LDL) and high-density lipoprotein (HDL); c) cholesterol-derived from the hydrolysis of stored cholesterol esters in the form of lipid droplets; and d) cholesterol interiorized from the plasma membrane, all this mechanisms implicating cell organels such as smooth endoplasmic reticuli, endosomes and of course mitochondria (Figure 1b). Although all three major steroidogenic organs (adrenal, testis and ovary) can synthesize cholesterol de novo under the influence of the tropic hormone, the adrenal and ovary preferentially utilize cholesterol supplied from plasma LDL and HDL via the LDL-receptor mediated endocytic pathway.

The use of LDL or HDL as the source of cholesterol for steroidogenesis appears to be species dependent; rodents preferentially utilize the SR-BI/selective pathway; this is a process in which cholesterol is selectively absorbed while the lipoprotein (mainly HDL) remains at the cell surface. The discovery of a specific receptor for this process (scavenger receptor class B, type I, known as SR-BI) has revolutionized the knowledge about the selective uptake pathway as a means of achieving cholesterol balance (Azhar et al., 2003).

Humans, pigs and cattle primarily employ the LDL/LDL-receptor endocytic pathway to meet their cholesterol need for steroid synthesis. In contrast, testicular Leydig cells under normal physiological conditions rely heavily on the use of endogenously synthesized cholesterol for androgen (testosterone) biosynthesis (Hu et al., 2010).

### 2.1. Ontogeny and sexual dimorphism

Steroidogenesis of gonadal sex hormones is by definition sexually dimorphic in hormonal action and also in regulation and temporal patterns of production.

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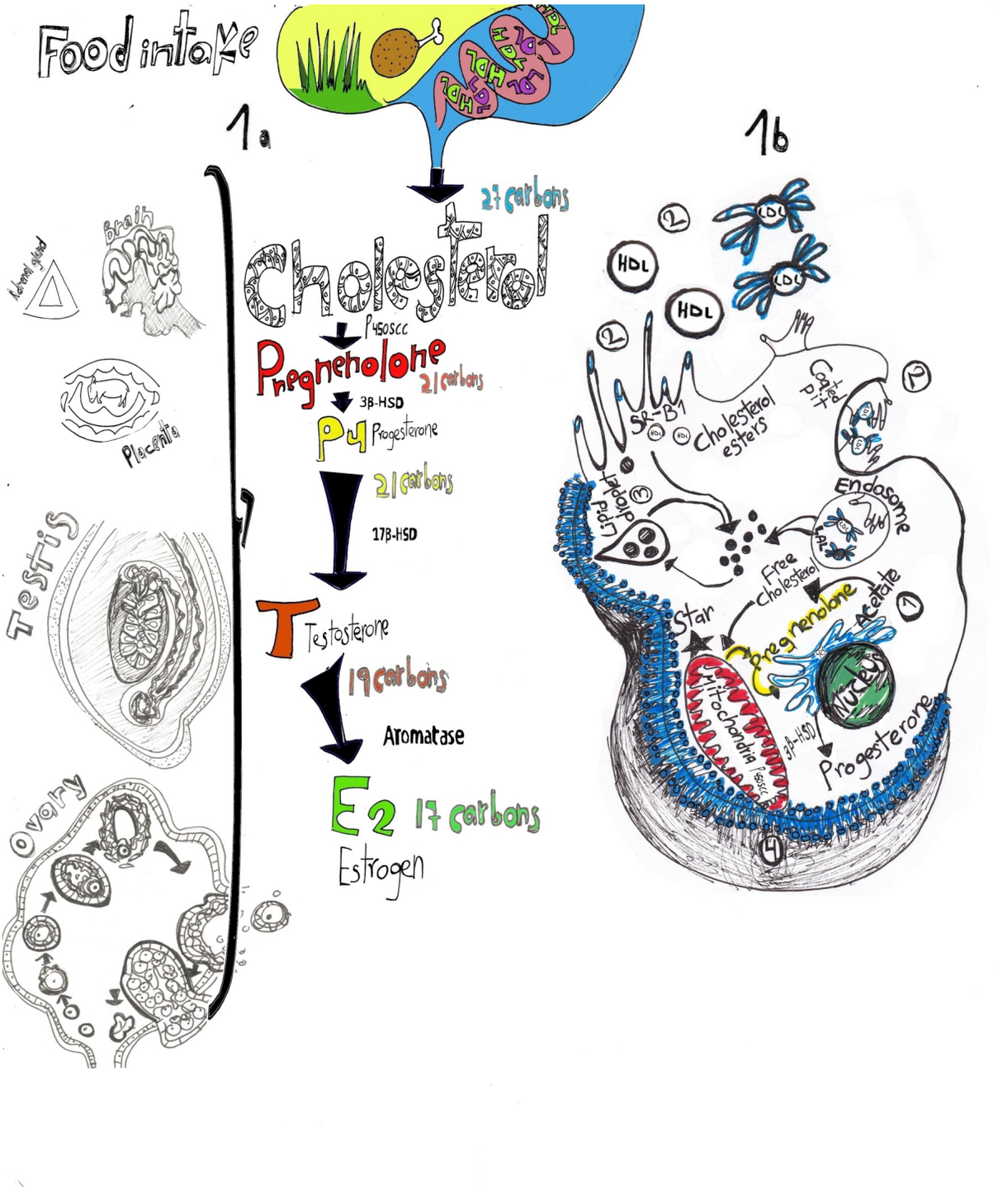


Ser: Smooth endoplasmic reticulum

**Figure 1.** Gonadal Steroids Synthesis Pathway. Modified from (Stocco, 2006; Senger, 2006). a) Steroidogenic tissues: adrenal gland, placenta, ovary, testis. Cholesterol from food intake is used (as LDL and HDL in plasma) by different cells in those tissues to synthesize the commune precursor: pregnenolone. The cascade continue with the androgens and estrogens production. b) Production of pregnenolone from four potential cholesterol sources: 1. synthesized *de novo* from acetate; 2. from plasma low-density lipoprotein (LDL) and high-density lipoprotein (HDL); 3. from the hydrolysis of stored cholesterol esters in the form of lipid droplets; and 4. Interiorized from the plasma membrane; cell organels implicated: smooth endoplasmic reticuli, endosomes and mitochondria

*2.1.1. Males*

The mesoderm-derived epithelial cells of the sex cords in developing testes become the Sertoli cells which will function to support sperm cell formation. A minor population of non-epithelial cells appears between the tubules by week 8 of human fetal development. These are Leydig cells. Soon after they differentiate, Leydig cells begin to produce androgens as mentioned before. In humans, Leydig cell populations can be divided into fetal Leydig cells that operate prenatally, and the adult-type Leydig cells that are active postnatally. Fetal Leydig cells are the primary source of testosterone and other androgens which regulate not only the masculinization of external and internal genitalia but also neuroendocrine function affecting behavioral and metabolic patterns.

Interestingly, adrenocortical and gonadal steroidogenic cells seem to share an embryonic origin in the coelomic epithelium, and they may exist as one lineage before divergence into the gonadal and adrenocortical paths. A common origin is also supported by the testicular adrenal rest tumours that are often found in male patients with congenital adrenal hyperplasia. Although much rarer, adrenal rests tumours have also been found in the ovary, also supporting the concept of a common origin of the steroidogenic cells. Those prenatal steroidogenic Leydig cells undergo degeneration and it is not well know which paracrine or endocrine factor(s) in the human fetal testis control this involution. Experiments on rodents have indicated that the regression of fetal Leydig cells occurs when plasma levels of LH remain high, suggesting that this gonadotropin cannot protect the cells from involution. It has been suggested that several factors – e.g. tumour growth factor b (TGFb), anti-Müllerian hormone (AMH), gonadotropin-releasing hormone (GnRH) –might play a role in fetal Leydig cell degeneration in rodents. TGFb is an attractive candidate for this purpose, since this factor is expressed by fetal Leydig cells during late fetal life and potently inhibits fetal Leydig-cell steroidogenesis in vitro.

It has been suggested that the development of human Leydig cells is triphasic and comprises fetal Leydig cells that function during the fetal period, neonatal Leydig cells that operate during the first year of life, and adult-type Leydig cells that appear from puberty onwards. This hypothesis is based on the triphasic developmental profile of plasma testosterone levels during human development.

All morphological modifications are accompanied by cellular growth and increasing expression of steroidogenic enzymes and LH receptors. These cellular events significantly enhance the capacity of mature Leydig cells to produce testosterone. Interestingly, reports in humans and experimental animals demonstrate that fully mature Leydig cells can dedifferentiate to previous stages of their development. These cellular events involve several morphological changes such as a reduction of the smooth endoplasmic reticulum and numbers of mitochondria, and impairment of T secretion. Paracrine control of Leydig cells steroidogenesis have been reported. Ghrelin appear to be appropriate markers for estimating the phase of Leydig-cell differentiation and the functional state of the cells. Leptin is another endocrine/paracrine factor that can modulate Leydig-cell steroidogenesis signalling transduction pathway(s) as a negative control in human Leydig cells. In a recent work we suggested a possible direct effect of leptin on calves gonads until the onset of puberty. The correlation

between the expression of leptin receptors (OBR) isoforms and their association with leptin and testosterone concentrations also indicated the complementary action of receptors and those hormones in peripubertal calves testis (Ruiz-Cortes and Olivera, 2010). Platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and endothelin and their receptors have been reported to be expressed in normal human Leydig cells, and have been suggested to play a role in the autocrine/paracrine regulation of human Leydig cell physiology (Svechnikov and Söder, 2008).

#### *2.1.2. Females*

In the ovary, the cellular contribution to steroidogenesis is very different from that in the testis, and both granulosa cells and theca cells contribute to steroidogenesis. In the testis supporting cell lineage gives rise to Sertoli cells which are nurse cells for spermatogenesis. For ovarian histogenesis, the supporting cell lineage gives rise to granulosa cells. Theca cells develop from stromal steroidogenic precursor cells outside the follicles and are ovarian counterparts of Leydig cells. The theca cells synthesize androgen in response to human chorionic gonadotropin, hCG and pituitary LH, but are not capable of producing estrogen since they lack expression of CYP19 aromatase, the enzyme converting androgen to estrogen. This enzyme is expressed by granulosa cells and these cells can produce estrogen and progesterone in response to LH and FSH stimulation. Thus, both theca cells and granulosa cells are required for estrogen synthesis by the ovary, and both gonadotropins (LH, FSH) are needed. These joint actions form the basis of the two cell, two-gonadotropin hypothesis for biosynthesis of estrogen. This is much more complex than the straight forward situation in the testis where Leydig cells produce androgen in response to LH (or hCG)(Svechnikov and Söder, 2008).

In the female, as in male, leptin excerts important action on steroidogenesis. We proved that Leptin, acting through STAT-3, modulates steroidogenesis in a biphasic and dose-dependent manner, and SREBP1 induction of StAR expression may be in the cascade of regulatory events in porcine granulosa cells (Ruiz-Cortes et al., 2003).

### 2.2. Androgens and anabolic steroids

#### *2.2.1. Androgens*

Scientists have studied androgens since the 18th century. Androgens are dubbed the male hormones mainly because males make and use more testosterone and other androgens than females. These steroid hormones confer masculinity by triggering and controlling body programs that govern male sexual development and physique. In females, androgens play more subtle roles (Tulane University).

The androgens, as paracrine hormones, are required by the Sertoli cells in order to support sperm production. They are also required for masculinization of the developing male fetus (including penis and scrotum formation)(Table 1). Under the influence of androgens, remnants of the mesonephron, the Wolffian ducts, develop into the epididymis, vas deferens and seminal vesicles. This action of androgens is supported by a hormone from Sertoli cells,

MIH (Müllerian inhibitory hormone), which prevents the embryonic Müllerian ducts from developing into fallopian tubes and other female reproductive tract tissues in male embryos. MIH and androgens cooperate to allow for the normal movement of testes into the scrotum. Two weak androgens, dehydroepiandrosterone and androstenedione are mostly synthesized in adrenal glands (in small amounts also in the brain). Androstenedione is converted into T mainly in testis Leydig cells and peripheral tissue, or aromatized into estradiol. Testosterone is metabolized by 5a-reductase in the potent androgen 5a-dihydrotestosterone and like androstenedione in estradiol by P450-aromatase (also called estrogen synthase) (Figure 1) (Michels and Hoppe, 2008).

In humans, the role of androgens with respect to breast growth and neoplasia was evaluated. Measurement of circulating sex steroids and their metabolites demonstrates that androgen activity is normally quite abundant in healthy women throughout the entire life cycle. Epidemiological studies investigating T levels and breast cancer risk have major theoretical and methodological limitations and do not provide any consensus. The molecular epidemiology of defects in pathways involved in androgen synthesis and activity in breast cancer holds great promise but is still in early stages. Clinical observations and experimental data indicate that androgens inhibit mammary growth and have been used with success similar to that of tamoxifen to treat breast cancer. Given these considerations, it is of concern that current forms of estrogen (E) treatment in oral contraceptives and for ovarian failure result in suppression of endogenous androgen activity. Thus, there is need for studies on the efficacy of supplementing both oral contraception and E replacement therapy with physiological replacement androgen, perhaps in a non aromatizable form, to maintain the natural E–androgen ratios typical of normal women (Dimitrakakis et al., 2002).

*2.2.2. Anabolic steroids*

Anabolic steroids are synthetic derivatives of testosterone and are characterized by their ability to cause nitrogen retention and positive protein metabolism, thereby leading to increased protein synthesis and muscle mass. Primary therapeutic use of testosterone is for replacement of androgen deficiencies in hypogonadism. These compounds are used for gynecologic disorders, anemia, osteoporosis, aging and treatment of delayed puberty in boys. Anabolic steroids have also been taken to improve athletic performance to enhance muscle development and to reduce body fat (Sevin et al., 2005).

According to surveys and media reports, the legal and illegal use of these drugs is gaining popularity. Testosterone restores sex drive and boosts muscle mass, making it central to 2 of society's rising preoccupations: perfecting the male body and sustaining the male libido. Testosterone has potent anabolic effects on the musculoskeletal system, including an increase in lean body mass, a dose-related hypertrophy of muscle fibers, and an increase in muscle strength. For athletes requiring speed and strength and men desiring a cosmetic muscle makeover, illegal steroids are a powerful lure, despite the risk of side effects. Recent clinical studies have discovered novel therapeutic uses for physiologic doses of anabolic-androgens steroids (AAS), without any significant adverse effects in the short term. In the wake of important scientific advances during the past decade, the positive and negative ef-

fects of AAS warrant reevaluation (Evans, 2004). In 1991 testosterone and related AAS were declared controlled substances. However, the relative abuse and dependence liability of AAS have not been fully characterized. In humans, it is difficult to separate the direct psychoactive effects of AAS from reinforcement due to their systemic anabolic effects. However, using conditioned place preference and self-administration, studies in animals have demonstrated that AAS are reinforcing in a context where athletic performance is irrelevant. Furthermore, AAS share brain sites of action and neurotransmitter systems in common with other drugs of abuse. In particular, recent evidence links AAS with opioids. In humans, AAS abuse is associated with prescription opioid use. In animals, AAS overdose produces symptoms resembling opioid overdose, and AAS modify the activity of the endogenous opioid system (Wood, 2008).

Antiandrogens prevent or inhibit the biological effects of androgens. They are often indicated to treat severe male sexual disorders such as paraphilias, as well as use as an antineoplastic agent in prostate cancer. They can also be used for treatment prostate enlargement, acne, androgenetic alopecia and hirsutism. The administration of antiandrogens in males can result in slowed or arrested development or reversal of male secondary sex characteristics, and hyposexuality (Sevin et al., 2005).

### 2.3. Estrogens and progestogens

Estrogens, or oestrogens, are a group of compounds named for their importance in the estrous cycle of humans and other animals. They are the primary female sex hormones. Natural estrogens are steroid hormones, while some synthetic ones are non-steroidal. Estrogen can be broken down into three distinct compounds: estrone, estradiol and estriol. During a mammal reproductive life, which starts with the onset of puberty and continues until andropause and /or menopause (in human), the main type of estrogen produced is estradiol. Enzymatic actions produce estradiol from androgens. Testosterone contributes to the production of estradiol, while the estrogen estrone is made from androstenedione. Phytoestrogens have analogous effects to those of human estrogens in serving to reduce menopausal symptoms, as well as the risk of osteoporosis and heart disease. In Animal husbandry (sheep and cattle) they may also have important physiological and sometimes deleterious reproductive effects as they are present in some pastures plants such as soybean, Alfalfa, red clover, white clover, subterranean clover, Berseem clover, birdsfoot trefoil and in native American legumes such as Vicia americana and Astragalus serotinus (Adams, 1995). Other estrogen containing foods Include: Anise seed, Apples, Baker's yeast, Barley, Beets, Carrots, Celery, Cherries, Chickpeas, Clover, Cucumbers, Dates, Eggs, Eggplant, Fennel, Flaxseed, Garlic, Lentils, Licorice, Millet, Oats, Olives, Papaya, Parsley, Peas, Peppers, Plums, Pomegranates, Potatoes, Pumpkin, Red beans, Rhubarb, Rice, Sesame seeds, Soybean sprouts, Soybeans, Split peas, Sunflower seeds, Tomatoes, Wheat, Yams.

Progestogens are characterized by their basic 21-carbon skeleton, called a pregnane skeleton (C21). In similar manner, the estrogens possess an estrane skeleton (C18) and androgens, an andrane skeleton (C19) (Figure 1). Progestogens are named for their function in maintaining pregnancy (pro-gestational), although they are also present at other phases of the estrous

and menstrual cycles. The progestogen class of hormones includes all steroids with a pregnane skeleton, that is, both naturally occurring and synthetic ones. Exogenous or synthetic hormones are usually referred to as progestins.

Progesterone is the major naturally occurring human progestogen. Progesterone (P4) is produced by the corpus luteum in all mammalian species. Luteal cells possess the necessary enzymes to convert cholesterol to pregnenolone (P5), which is subsequently converted into P4. Progesterone is highest in the diestrus phase of the estrous cycle as is going to be explained.

*2.3.1. Estradiol*

Estradiol (E2 or 17β-estradiol, also oestradiol) is a sex hormone. Estradiol has 17 carbons (C17) and 2 hydroxyl groups in its molecular structure, estrone has 1 (E1) and estriol has 3 (E3). Estradiol is about 10 times as potent as estrone and about 80 times as potent as estriol in its estrogenic effect. Except during the early follicular phase of the menstrual cycle, its serum levels are somewhat higher than that of estrone during the reproductive years of the human female. Thus it is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity. During menopause, estrone is the predominant circulating estrogen and during pregnancy estriol is the predominant circulating estrogen in terms of serum levels. Estradiol is also present in males, being produced as an active metabolic product of testosterone. The serum levels of estradiol in males (14 - 55 pg/mL) are roughly comparable to those of postmenopausal women (< 35 pg/mL). Estradiol *in vivo* is interconvertible with estrone; estradiol to estrone conversion being favored. Estradiol has not only a critical impact on reproductive and sexual functioning, but also affects other organs, including the bones (Table 1).

There is scientific literature that may be relevant about the use of estradiol from the point of view of food safety. In cattle for example Estradiol benzoate (10-28 mg) or estradiol-17ß (estradiol; 8-24 mg) is administered (orally) to cattle to increase the rate of weight gain (i.e. growth promotion) and to improve feed efficiency. Estradiol valerate is also administered by subcutaneous or intramuscular injection to synchronize estrus in cattle. Estradiol is generally considered to be inactive when administered orally due to gastrointestinal and/or hepatic inactivation.

Circulating estradiol, like T, is bound to sex hormone-binding globulin (SHBG, in Figure 4) and, to a lesser extent, serum albumin. Only 1-2% of circulating estradiol is unbound; 40% is bound to SHBG and the remainder to albumin. Plasma SHBG is secreted from the liver; a similar, non-secretory form is present in many tissues, including reproductive tissues and the brain.

Urinary and faecal metabolites of estrogens in animals and humans have been studied for use as possible indicators of risk for hormone-dependent cancers or for infertility. There is at present no consensus about the importance of specific metabolites or metabolite ratios as prognostic factors, with the possible exception of estriol as a marker of the well-being of the feto-placental unit (World Health Organization International Programme on Chemical Safety, 2000).

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Estrogens have been isolated from testes of stallion, bulls, boars, dogs and men. Estrogens may play a role in the pathogenesis of prostatic hyperplasia common in aged dogs, and estrogens receptors are present in prostatic urethra and prostatic glands of dogs. Estrogens like androgens, are transferred from testicular vein to the testicular artery. In several species, levels of estrogens in the blood of testicular artery are consistently higher than the levels in systemic blood. The mechanisms involved in the transfer of estrogens from vein to artery in the pampiniform plexus and its physiology role are not clear. Estrogens may be playing important role in regulating the pituitary-gonadal axis. In several species, estrogens inhibit Leydig cell secretion of testosterone (Pineda, 2003) as it will be mentioned.

| Name of Steroid (abbrev.) | Steroidogenic Tissues | Target Tissue (male and female) | Physiological functions (male and female) |
|---|---|---|---|
| Estradiol (E2) | Granulosa cells of follicle<br>Placenta<br>Sertoli cells of testis | Brain, hypothalamus<br>Bones<br>Entire female reproductive tract and mammary gland | Sexual behavior (male and female)<br>Secondary female sex characteristics<br>GnRH regulation, Ovulation<br>Elevated secretory activity of the entire female tract<br>Enhanced uterine motility<br>Regulation of cardiovascular physiology<br>Bone integrity and neuronal growth |
| Progesterone (P4) | Luteinized/luteal cells<br>Placenta<br>Adrenal | Hypothalamus<br>Uterine endometrium, myometrium<br>Mammary gland<br>Leydig cells | Follicular growth and ovulation<br>Endometrial secretion<br>Inhibits GnRH release<br>Inhibits reproductive behavior<br>Promotes maintenance of pregnancy |
| Testosterone (T) | Leydig cells of testis<br>Theca interna cells of ovary | Accesory sex glands (male)<br>Tunica dartos of scrotum<br>Seminiferous epithelium<br>Skeletal muscle<br>Brain (female)<br>Granulosa cells | Anabolic growth (male), Increase muscle mass<br>Promotes spermatogenesis<br>Promotes secretion of accessory sex glands<br>Substrate for E2 synthesis (female)<br>Secondary sex characteristics<br>Decrease risk of osteoporosis |

**Table 1.** Sex steroids: Source, Target tissues and Physiological Functions. Modified from (Hu et al., 2010; Senger, 2006)

*2.3.2. Progesterone*

Progesterone, also known as P4 (pregn-4-ene-3,20-dione), is a C-21 steroid hormone involved in the female menstrual/estral cycle, pregnancy and embryogenesis of humans and other species. Progesterone is produced in the ovaries, the adrenal glands (suprarenal), and, during pregnancy, in the placenta. Progesterone is also stored in adipose (fat) tissue. Progesterone is synthesized by the ovarian corpus luteum, but during pregnancy the main source of P4 is the placenta as in woman,mare and ewe; in cow, the time of placenta takeover is 6-8months of pregnancy. In other species (goat, sow, queen, bitch,rabbit, alpaca,camel, llama) there is no placenta P4 production at all, the ovarian CL is in charge of the entire P4 for gestation. In mammals, P4, like all other steroid hormones, is synthesized from pregnenolone, which in turn is derived from cholesterol. Androstenedione can be converted to testosterone, estrone and estradiol (Figure 1)(Wikipedia). Important functions of P4 are (1) inhibition of sexual behavior; (2) maintenance of pregnancy by inhibiting uterine contractions and promoting glandular development in the endometrium; and (3) promotion of alveolar development of the mammary gland. The synergistic actions of estrogens and progestins are notable in preparing the uterus for pregnancy and the mammary gland for lactation (Table 1).

In at least one plant, *Juglans regia*, progesterone has been detected. In addition, progesterone-like steroids are found in *Dioscorea mexicana*. It contains a steroid called diosgenin that is taken from the plant and is converted into progesterone. Diosgenin and progesterone are found in other *Dioscorea* species as well.

The switch from the principal steroid product of the maturing follicle (estrogens) to that of the developing and mature corpus luteum (P4) is one of the amazing hallmarks of the ovary sex steroids production occurring during luteinization as described later.

Of interest, we have reported that during the differentiation of granulosa cells into luteal cells *in vitro*, it exists an inverse modulation between the expression of LH receptors (LHR) and the concentration of LH, and this expression of LHR could be regulated by P4 produced by luteinized granulosa cells (Montaño et al., 2009).

## 3. Functional organization of the hypothalamic-pituitary-gonadal axis: sex steroids control of reproduction

Gonadal secretory activites involve two special cell types responsive to FSH and LH. Ovarian granulosa cells and testicular Leydig cells are responsive primarily to LH and synthesize androgens. Ovarian thecal cells and testicular Sertoli cells as well as Leydig cells respond to FSH with conversion of androgens into estrogens (P450aromatase activity). FSH also stimulates Sertoli cells to synthesize inhibin, activin, and other local bioregulatory factors (Norris, 2007).

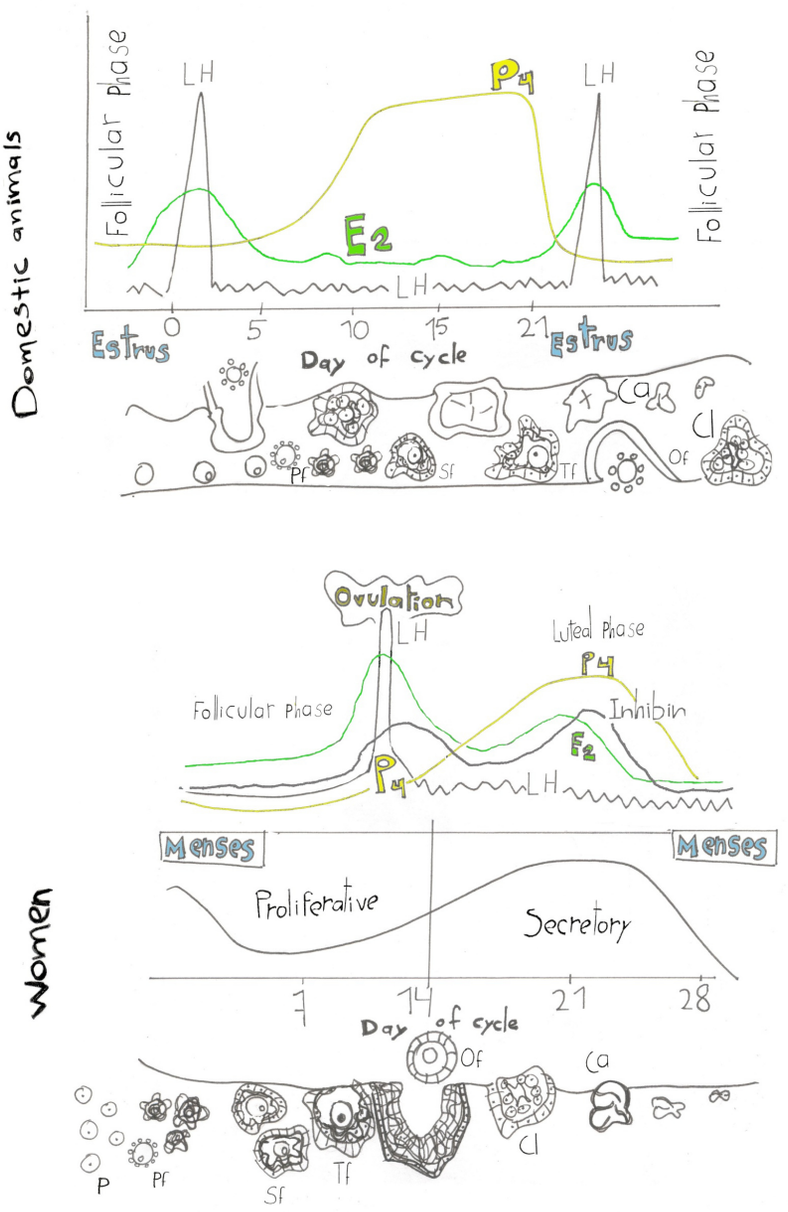
### 3.1. Gonadal steroids and female reproductive cyclicity

Anatomically in the female hypothalamus, there are two GnRH neurons centers. The first, the surge center, consists of three nuclei called the preoptic nucleus, the anterior hypothalamic area and the suprachiasmatic nucleus. This center releases basal levels of GnRH until it receives the appropriate positive stimulus. This stimulus is known to be a threshold level of estrogen in the absence of P4. When the estrogen concentration in the blood reaches a certain level, a large quantity of GnRH is released from the terminals of neurons, the cells bodies of which are located in the surge center. In natural condition, the preovulatory surge of GnRH occurs only once during the estrous or menstrual cycle. The second, the tonic center, releases small episodes of GnRH in a pulsatile fashion similar to a driping faucet. This episodic release is continuous and throughout reproductive life and during the entire estrous cycle (Senger, 2006).

The female in various species have two important periods that mark the reproductive cycle: follicular and luteal phases. The follicular phase begins after luteolysis that causes the decline in P4. Gonadotropins (FSH and LH) are therefore produced and cause follicles to produce E2. The follicular phase is dominated by E2 produced by ovarian follicles and ends at ovulation. The luteal phase begins after ovulation and includes the development of corpus luteum that produces P4, and luteolysis that brought about by prostaglandin F2$\alpha$. In women, the follicular phase is divided into menses and proliferative period (5 and 9 days respectively); luteal phase is the secretory phase (14 days). In domestic animals, the follicular phase is divided in pro-estrus (2 days) and estrus (1 day), and the luteal phase in metestrus (4 days) and diestrus (14 days). At the pro-estrus, as P4 drops, FSH and LH increase together in response to GnRH. FSH and LH cause the production of E2 by ovarian follicles (Figure 2). When recruited follicles develop dominance, they produce E2 and inhibin that suppresses FSH secretion from the anterior lobe of the pituitary. Thus, FSH does not surge with the same magnitude as LH. The pre-ovulatory surge of GnRH is controlled by high E2 and low P4. In mammals, including humans, E2 in the presence of low P4 exerts a differential effect on GnRH. Thus, E2 in low concentrations causes a negative feedback (suppression) on the preovulatory center. That is, low estrogen reduces the level of firing GnRH neurons in the preovulatory-surge center. However, when E2 levels are high (estrus), as they would be during the mid-to late follicular phase (figure 2), the preovulatory center responds dramatically by releasing large quantities of GnRH. This stimulation in response to rising concentrations of E2 is referred to as positive feedback. During the middle part of the cycle, when E2 levels are low and P4 is high (metestrus, diestrus), there is negative feedback on the preovulatory center, thus preventing high amplitude pulses of GnRH. Interesting, when comparing human vs. other mammals, the P4 does not influence sexual receptivity but in domestic animals, those high levels of P4 inhibit it (Senger, 2006) (Figure 2).

As reviewed by Murphy, luteinization is a remarkable event involving cell proliferation, cell differentiation, and tissue remodeling that is unparalleled in the adult mammal. It comprises two major processes: (a) the terminated proliferation plus rapid hypertrophy and differentiation of the steroidogenic cells of follicle into the luteal cells of the CL. Luteinization is both a qualitative and quantitative change because the mammalian CL produces up to 100-fold greater amounts of steroid (P4) than the follicle. Luteolysis results in cessation of P4

production, in structural regression to forma corpus albicans and into a follicular development and entrance into a new follicular phase.




P: Primordial follicle, PF: Primary follicle, SF: Secondary follicle, TF: Tertiary follicle, OF: Ovulatory follicle, Cl: Corpus luteum, Ca: Corpus albicans

**Figure 2.** Female cyclicity and gonadal steroids. Modified from (López et al., 2008; Senger, 2006) The two types of reproductive cycles are the estrus and the menstrual cycles. Each cycle consists of a follicular and a luteal phase. The follicular phase is dominated by the hormone E2 from ovarian follicles. E2 causes marked changes in the female tract for pregnancy. Anestrus stands for periods of time when estrous cycles cease. Pregnancy, season of the year, lactation, forms of stress and pathology cause anestrus. Amenorrhea refers to the lack of menstrual periods and is caused by many of the same factors that cause anestrus. A menstrual cycle consists of the physiological events that occur between successive menstrual periods (about 28 days). No endometrial sloughing (menstruations) occurs in animal with estrous cycles.Lutealphase is dominated by P4 from corpus luteum.

As the main steroid produced during luteal phase is the P4 it is important to mention about the manipulation of the estrous and menstrual cycles by exogenous administration of P4. It serves indeed as an "artificial corpus luteum" (ear subcutaneous implants or intravaginal devices). Exogenous P4 suppresses estrus and ovulation. When this exogenous P4 is removed or withdrawn, the animal will enter pro-estrus and estrus within 2 to 3 days after removal. This application is intended to increase the convenience of artificial insemination programs and to facilitate fertility in domestic husbandry animal (improving pregnancy rates). In contrast, the use of exogenous P4 in humans (oral, transdermal,injectable, implants) is intended to block ovulation and minimize pregnancy probability (contraception)(Senger, 2006).

### 3.2. Gonadal steroids and spermatogenesis

Upon stimulation by LH, the Leydig cells of the testes produce androgens. Dihydrotestosterone is found in high enough concentration in peripheral tissue to be of functional importance. Functions of T, as states before, include (1) development of secondary sex characteristics; (2) maintenance of the male duct system; (3) expression of male sexual behavior (libido); (4) function of the accessory glands; (5) function of the tunica dartos muscle in the scrotum; and (6) spermatocytogenesis. The role of T in regulating the release of hypothalamic and gonadotropic hormones is similar to that described for P4 in the female. High concentrations of T inhibit the release of GnRH, FSH, and LH, a negative feedback control. Conversely, when T concentrations are low, higher levels of GnRH, FSH, and LH are released. Thus, reciprocal action of T with the hypothalamic and gonadotropic hormones is necessary for regulation of normal reproduction in the male (Figure 3)(Gyeongsang National University). Luteinizing hormone acts on the Leydig cells within the testes. These cells are analogous to the cells of the theca interna of antral follicles in the ovary. They contain membrane bound receptors for LH. When LH binds to their receptors, Leydig cells produce P4, most of which is converted to T. The production of T takes place by the same intracellular mechanism as in the female. The Leydig cells synthesize and secrete T less than 30 minutes after the onset of an LH episode (Figure 3). This T secretion is short and pulsatile, lasting for a period of 20 to 60 minutes. It is believed that pulsatile discharge of LH is important for two reasons. First, high concentration of T within the seminiferous tubule is essential for spermatogenesis (Senger, 2006). Second, Leydig cells become unresponsive to sustained high levels of LH believed to be caused by reduction in the number of LH receptor. In fact, continual high concentrations of LH result in reduced secretion of T. Intratesticular levels of T are 100-500 times higher than that of systemic blood. However, testicular T is diluted over 500 times when it reaches the peripheral blood (Senger, 2006). This dilution added to a short half-life of the T (here, there is considerable variation in the half-life of testosterone as reported in the literature, ranging from 10 to 100 minutes; it is metabolized in the liver) keep systemic concentrations well below that which would cause down-regulation of the GnRH/LH feedback. The role of the pulsatile nature of T is not fully understood. It is believed that chronically high systemic concentrations of T suppress FSH secretion. Sertoli cells function is FSH dependent. Thus, their function is compromised when FSH is reduced. The periodic reduction in T allows the negative feedback on FSH to be removed. But the exact role of this FSH diminution it is not clear as well as the physiological role of paracrine/

autocrine inhibin effects within the testis has not been clarified. While the $\alpha$ subunit knockout mouse model suggests that this protein protects against the development of testicular tumours, there is no evidence for a physiological role of paracrine/autocrine inhibin signalling on spermatogenesis or steroidogenesis (de Kretser et al., 2001). Sertoli cells also produce inhibin that, as in the female, suppresses FSH secretion from the anterior lobe of the pituitary. The physiologically important hormone that exerts tonic negative feedback upon FSH secretion in men is inhibin B (Illingworth et al., 1996). Inhibin and androgen binding protein are produced by Sertoli cells under the influence of FSH. As in the female, inhibin selectively inhibits the release of FSH while not affecting the release of LH. Androgen binding protein binds T, making it available for its functions in spermatozoa production.

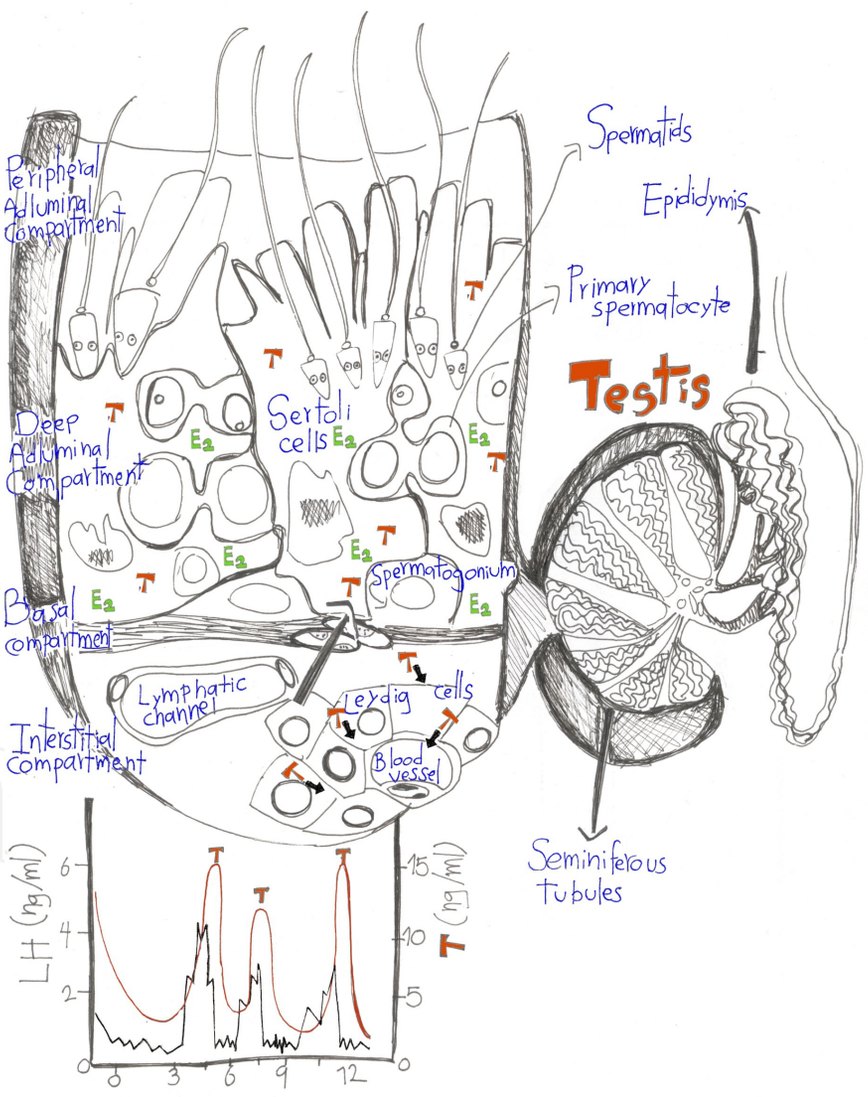



**Figure 3.** Spermatogenesis and steroids. Modified from (Senger, 2006). There is a pulsatile discharge of LH. Leydig cells produce important concentrations of testosterone (T).High concentration of T within the seminiferous tubule, essential for spermatogenesis. Sertoli cells aromatize T from Leydig cell into E2.

Under the influence of FSH the Sertoli cells convert T to E2 and other estrogens (Figure 3). The stallion and the boar secrete large amount of E2 but since they are secreted as molecules with low physiologic activity they seem to be of little consecuence. Sertoli cells convert T to E2 utilizing a mechanism identical to the granulosal cell of the antral follicle in the female (Senger, 2006). The exact role of E2 in male reproduction it is not clear. The finding of both aromatase and E2 receptors (ERs) in the developing fetal testis implies a possible involvement of estrogens in the process of differentiation and maturation of developing rodent testis from an early stage of morphogenesis, probably ERβ having a major role than ER$\alpha$ (Luconi et al., 2002; Rochira et al., 2005). Also, T and E2 in the blood act on the hypothalamus and exert a negative feedback on GnRH and, in turn, LH and FSH are reduced.

### 3.3. Sex steroids molecular pathways in target tissues

Steroid hormones regulate cellular processes by binding to membrane, intracellular and/or nuclear receptors that, in turn, interact with discrete nucleotide sequences to alter gene expression. Because most steroid receptors in target cells are located in the cytoplasm, they need to get into the nucleus to alter gene expression. This process typically takes at least 30 to 60 minutes. In contrast, other regulatory actions of steroid hormones are manifested within seconds to a few minutes. These time periods are far too rapid to be due to changes at the genomic level and are therefore termed nongenomic or rapid actions, to distinguish them from the classical steroid hormone action of regulation of gene expression. The rapid effects of steroid hormones are manifold, ranging from activation of mitogen-activated protein kinases (MAPKs), adenylyl cyclase (AC), protein kinase C and A (PKC,PKA), and heterotrimeric guanosine triphosphate-binding proteins (G proteins) (in Figure 4 and 5). In some cases, these rapid actions of steroids are mediated through the classical steroid receptor that can also function as a ligand-activated transcription factor, whereas in other instances the evidence suggests that these rapid actions do not involve the classical steroid receptors. One candidate target for the nonclassical receptor-mediated effects are G protein-coupled receptors (GPCRs), which activate several signal transduction pathways. One characteristic of responses that are not mediated by the classical steroid receptors is insensitivity to steroid antagonists, which has contributed to the notion that a new class of steroid receptors may be responsible for part of the rapid action of steroids. Evidence suggests that the classical steroid receptors can be localized at the plasma membrane, where they may trigger a chain of reactions previously attributed only to growth factors. Identification of interaction domains on the classical steroid receptors involved in the rapid effects, and separation of this function from the genomic action of these receptors, should pave the way to a better understanding of the rapid action of steroid hormones (Cato et al., 2002; Simoncini et al., 2004) (Figure 4 and 5).

#### *3.3.1. Androgens*

The biological activity of androgens is thought to occur predominantly through binding to intracellular androgen-receptors, a member of the nuclear receptor family, that interact with specific nucleotide sequences to alter gene expression. This genomic-androgen effect typically takes at least half an hour. In contrast, the rapid or non-genomic actions of androgens are

manifested within in seconds to few minutes. This rapid effect of androgens are manifold, ranging from activation of G-protein coupled membrane androgen receptors or sex hormone-binding globulin receptors, stimulation of different protein kinases, to direct modulation of voltage- and ligand gated ion-channels and transporters. The physiological relevance of these non-genomic androgen actions has not yet been determined in detail. However, it may contribute to modulate several second messenger systems or transcription factors, which suggests a cross-talk between the fast non-genomic and the slow genomic pathway of androgens (Michels and Hoppe, 2008) (Figure 4).

The rapid actions of androgens are mediated by direct binding to the target protein (e.g., ion-channel) or by a specific association to different receptors. The non-genomic androgen action based on receptor level can be mediated by at least three androgen-binding proteins, the classical intracellular androgen receptor, the transmembrane androgen receptor and the transmembrane sex hormone-binding globulin receptor. For both transmembrane receptors, the non-genomic effect is converted via a G-protein coupled process, whereas binding to intracellular androgen receptors may lead to an activation of several cytosolic pathways. All rapid androgen actions are predominantly mediated by second messenger signaling (especially Ca2+) and phosphorylation events, including different intracellular signal routes, e.g., PKA, MAPK, phospholipase:PLC, phosphatidylinositol-3 kinase:PI-3K, steroid receptor coactivator:Src pathways. Although some studies implicated benefits of the non-genomic androgen actions on the cardiovascular and neuropsychiatric systems, more detailed research and clinical studies are still required (Michels and Hoppe, 2008).

Increasing evidence suggests that nongenomic effects of testosterone and anabolic androgenic steroids (AAS) operate concertedly with genomic effects. Classically, these responses have been viewed as separate and independent processes, primarily because nongenomic responses are faster and appear to be mediated by membrane androgen receptors, whereas long-term genomic effects are mediated through cytosolic androgen receptors regulating transcriptional activity. Numerous studies have demonstrated increases in intracellular Ca2+ in response to AAS. These Ca2+ mediated responses have been seen in a diversity of cell types, including osteoblasts, platelets, skeletal muscle cells, cardiac myocytes and neurons. The versatility of Ca2+ as a second messenger provides these responses with a vast number of pathophysiological implications. In cardiac cells, testosterone elicits voltage-dependent Ca2+ oscillations and inositol-1,4,5-triphosphate receptors:IP3R mediated Ca2+ release from internal stores, leading to activation of MAPK and the serine/threonine protein kinase regulating cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription: mTOR. In neurons, depending upon concentration, testosterone can provoke either physiological Ca2+ oscillations, essential for synaptic plasticity, or sustained, pathological Ca2+ transients that lead to neuronal apoptosis. It was proposed that Ca2+ acts as an important point of crosstalk between nongenomic and genomic AAS signaling, representing a central regulator that bridges these previously thought to be divergent responses (Vicencio et al., 2011).

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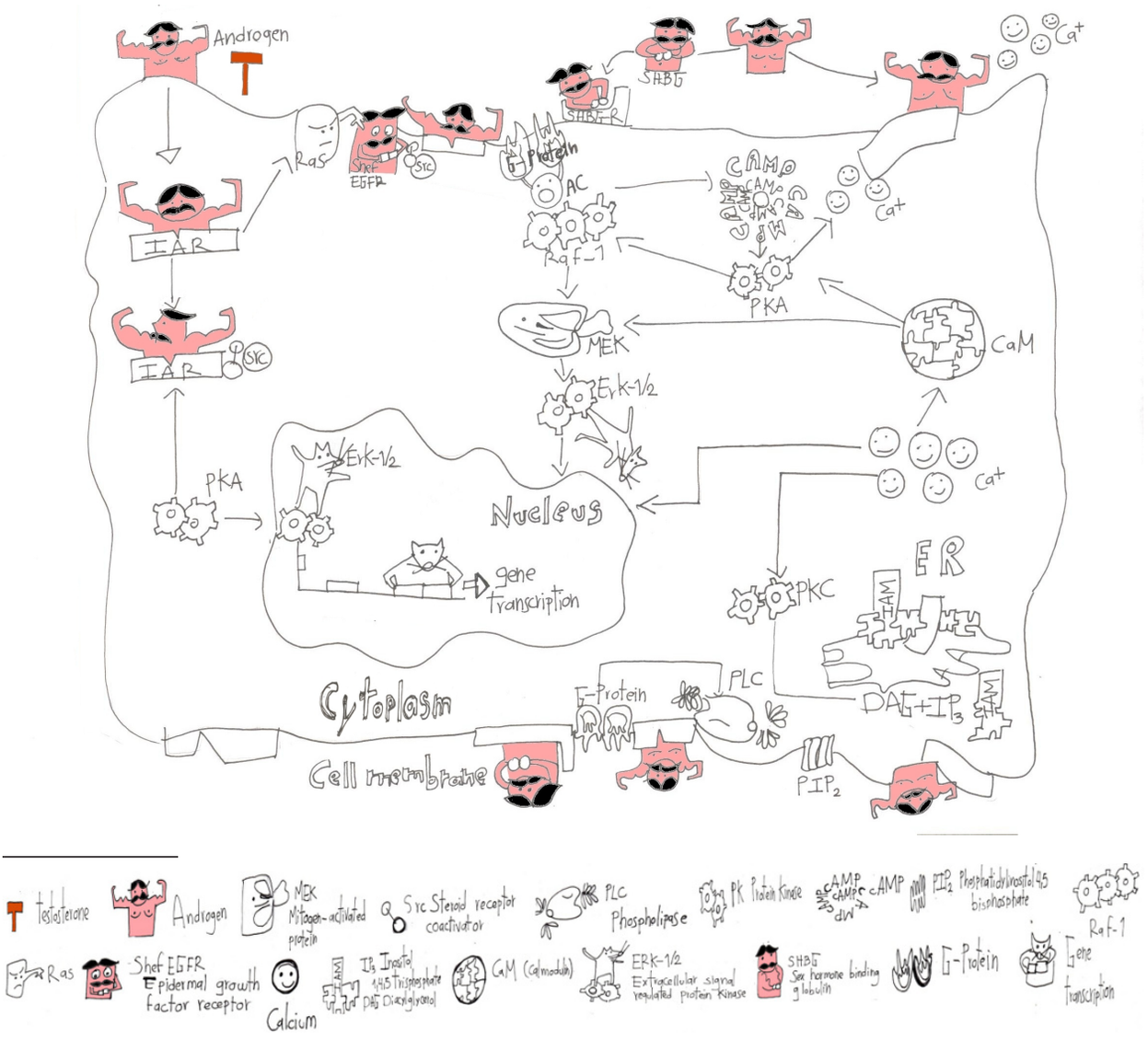


**Figure 4.** Actions and pathways of androgens. Modified from (Michels and Hoppe, 2008). The rapid effects of steroid hormones are mediated by he activation of mitogen-activated protein kinases (MAPKs), adenylyl cyclase (AC), protein kinase C and A (PKC,PKA), and heterotrimeric guanosine triphosphate-binding proteins (G proteins). The cross-talk between the fast non-genomic and the slow genomic pathway by androgens binding to their internal androgens receptors (IAR) is mediated in part by intracellular Ca2+.

### *3.3.2. Estrogens*

In 2009 Chariditi et al. described four main pathways of estrogen receptors (ERs) alpha (a) and beta (b) signaling as a matter of "sophisticated" control systems necessary to obtain a tight equilibrium in estrogen action and regulation of ER expression in tissues and cells (Charitidi et al., 2009).

The first well known molecular mechanism is the classic ligand dependent pathways. Estrogen receptors are kept inactive in the nucleus and cytoplasm of the cell forming a complex with various heat shock proteins (hsp) that act as chaperones when the cell is not exposed to estro-

gens. Such proteins are hsp90, hsp70 and hsp56 and by forming a complex with the ERs they are believed to prevent them from binding to their response elements EREs, but also keep them capable of binding to their ligands (estrogens) with high affinity. When the estrogens diffuse across the cell and nuclear membrane they interact with the inactive form of the ERs and separate them from the hsp-complex. ERs are now activated and can form homodimers and to a lesser extent heterodimers to bind to their estrogens EREs. The EREs are commonly located in the promoter regions of estrogen target genes and make it possible for the ERs to specifically bind to the DNA and regulate transcription either as enhancers or repressors.

Once the complex of the activated ERs together with co-activator proteins (such as ligand-dependent activation function-1 and 2: AF-1 and AF-2) is bound to the ERE it can either up- or down-regulate the expression of the target gene. This is decided by whether the ERE is ''positive" or ''negative" in the particular cell type for the ERs as well as by the cellular milieu (Figure 5).

The second molecular mechanism is the ligand independent. It is possible that the ERs get activated even in the absence of their ligands with the aid of intracellular second messengers. Growth factors are able to activate MAPKs and they subsequently become phosphorylated and thus activate the ERs. This ligand-independent ER activation is still dependent on AF-1. Another intracellular path that can lead to ER activation in the absence of ligands is via cAMP, a second messenger for G-protein coupled receptors and activates the PKA pathway. AF-2 is needed for cAMP activation of ERs. In this type of ligand-independent activation of ERs, growth factors and second messengers take over estrogens part to induce/elicit the same response from ERs (Figure 5).

The third signaling pathway is the ERE independent one. Estrogens exert their actions through the two ERs but also through other transcription factors. In this case the ligand-activated ERs do not bind to their EREs but anchor instead to other transcription factors directly bound to DNA in their specific response elements. In this mechanism ERs act more as co-regulators than actual transcription factors (activating protein-1 (AP-1), (Fos/Jun) or the stimulating protein-1 (Sp1)). Thus this, pathway is also referred to as transcription factor cross-talk (Figure 5)). Furthermore, the two ERs differ in their capacity to interact with different transcription factors. For example in the presence of 17beta-estradiol, ERa induces AP-1 driven gene transcription, while ERb has an inhibitory effect. This contrasting transcriptional activity is another example of the opposing actions of each ER.

The last mechanism is the non-genomic plasma-membrane pathway. The above mentioned mechanisms include the relatively long processes of gene transcription and mRNA translation and are thus insufficient to explain the short-term effects of estrogens that are found. Intracellular pathways that increase intracellular calcium, cAMP, or the phosphorylation of the cAMP response element binding protein (CREB), can result in an instantaneous response of the cell. This pathway does not require transcription of genes via the ERs and is referred to as non-genomic mechanisms of estrogen action, similar to the non-genomic pathways of androgens (Charitidi et al., 2009) (Figure 5).

In adults, the interaction of estrogen genomic and nongenomic mechanisms may act to maintain physiology or signal transduction pathways as hormone levels fluctuate across the estrus cycle. As such, a disruption of the hormone/receptor system through a loss of hor-

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mone, decreased receptor expression, or uncoupling of receptor-transcriptional activity due to chronically elevated estrogen levels, would contribute to age-related changes that underlie the progressive senescence of physiological processes. Treatments designed to increase ER activity around the time of menopause, such as cyclic estrogen replacement, may be more beneficial than chronic hormone replacement (Foster, 2005).

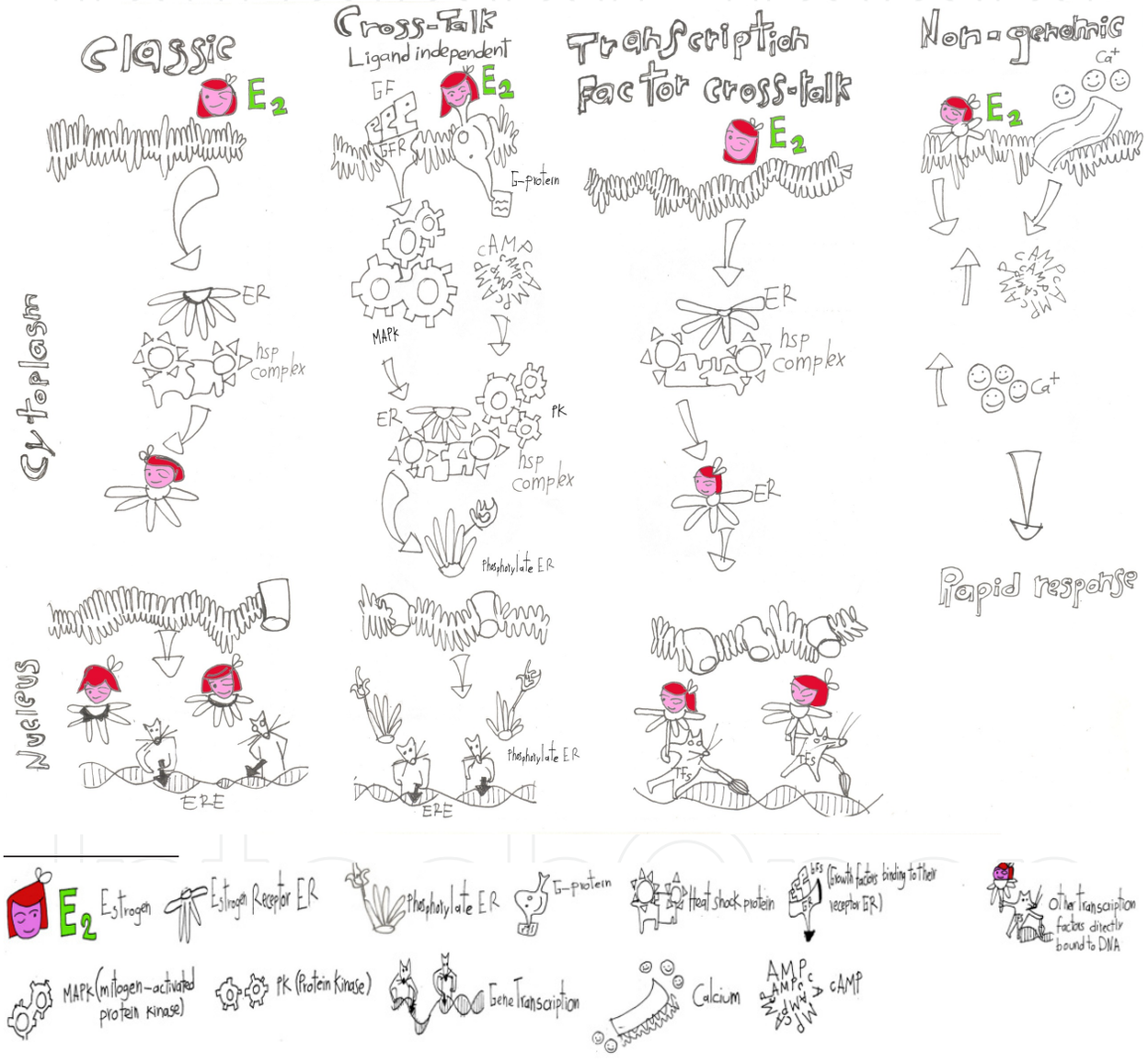


**Figure 5.** Actions and pathways of estrogens. Modified from (Charitidi et al., 2009). Four molecular mechanisms of E2 signaling in target cells. The first is the classic ligand dependent pathways. Estrogen receptors (ER) are liberated from heat shock proteins complex (hsp) and can continue their nuclear-DNA effect. The second is the ligand independent. It is possible that the ERs get activated even in the absence of their ligands with the aid of intracellular second messengers. The third is the ERE independent. In this case the ligand-activated ERs do not bind to their EREs but anchor instead to other transcription factors. The fourth is the non-genomic plasma-membrane pathway and does not require transcription of genes via the ERs. Besides those well documented genomic and non- genomic molecular pathways, it is important to mention the epigenetic regulation.

Recently, it was published a review about the overlapping nongenomic and genomic actions of thyroid hormone and estrogens and androgens. Authors concentrate on the tumor cell model, where, for example, estrogens and thyroid hormone have similar MAPK-dependent proliferative actions and where dihydrotestosterone also can stimulate proliferation. Steroids and thyroid hormone have similar anti-apoptotic effects in certain tumors; they also have overlapping or interacting nongenomic and genomic actions in heart and brain cells.

Their possible clinical consequences seem of crutial importance for the potential endocrine therapy targeting steroids receptors directly or indirectly (hormone or protein with overlapping effects) as reported for breast cancer and the nuclear and citoplasmic estrogen receptor and aromatase (Davis et al., 2011; Levin and Pietras, 2008).

Estradiol epigenetic effects have been reported with results providing evidence for mitotic regulation in follicle development by estrogen and demonstrate a previously undiscovered mechanism for induction of cell proliferation in ovarian and mammary gland cells. This epigenetic mark is induced by both FSH and 17beta-estradiol (E2), acting independently. E2-induced H3 phosphorylation fails to occur in mice with inactivated alpha-isoform of the nuclear estrogen receptor. E2 induction of histone phosphorylation is attenuated by cell cycle inhibition. Further, E2 induces the activity of the mitotic kinase, Aurora B, in a mammary tumor cell model where mitosis is estrogen receptor-alpha dependent (Ruiz-Cortes et al., 2005).

### 3.4. Reproductive moments and steroids

#### *3.4.1. Puberty*

Sex steroids regulation of the initiation of puberty was reported since 1979 in murine studies. Immature female rats presented evidence of oestrogen secretion by day 32 of life and an increased sensitivity of the pituitary to LHRH by day 34. These data suggested that in addition to the increased release of GnRH during puberty, a sex steroid induced alteration in the pituitary's responsiveness to GnRH may also be a significant contributory factor in the increase in secretion of gonadotropins at puberty. The stimulatory effect appeared to be related both to the quantity of sex steroid and the challenging dose of GnRH. These studies show that in addition to changes in sensitivity at the level of the hypothalamus, the CNS and gonads steroid and GnRH modulation of the response of the pituitary gland, are important events in the onset of puberty (Mahesh and Nazian, 1979).

Puberty is associated with an increasing production of androgenic steroids. Adrenal androgen formation (adrenarche), may precede gonadal testosterone synthesis. Both adrenal and gonadal androgens exert their biological effects via the androgen receptor, a nuclear transcription factor modulating a specific transcription regulation of largely unknown genes. During puberty, virilizing actions such as genital enlargement and sexual hair growth can be distinguished from anabolic action such as the gain in muscle strength and general changes in body composition. Furthermore, androgens play a major role in the initiation and maintenance of spermatogenesis. Thus, different androgenic steroids play an important role in the process of puberty (Hiort, 2002)(Table 2).

Male infants have a surge in T levels during the first few months of life. These levels fall to quite low (but greater than in female infants and children) until the pubertal rise. Nighttime elevations in serum T concentration are detectable even before the onset of the external signs of pubertal development following the sleep-entrained rises in serum LH. The daytime levels rise later as the testis volume increases.Testosterone is a substrate for 5-a reductase (conversion to dihydrotestosterone) and for aromatase (conversion to estradiol). The effects on muscle are likely in part due directly to T and indirectly to E2 because of the marked increase in growth hormone-GH and IGF-I levels due to an action of E2 on the hypothalamus and pituitary (Rogol, 2002).

In domestic animals, Senger and his team very appropriately mentioned in his book how the "story on the onset of puberty is not complete". It is about many factors that may be controlling this important physiological process of acquiring reproductive and productive competence. This capability is influenced by achieving the appropriated energy metabolism/body size and appropriated exposure to external modulators such as photoperiod (goat, sheep, horse), size of social groups (pig, cow) and the presence of the male (cow, goat). Genetics of the animal likely play a role in how these cues are generated within the animal (metabolic signals) and /or perceived (external cues, metabolic signals). The exact mechanisms that enable E2 to control GnRH secretion by the hypothalamus during the peripubertal period are still unknown even if since 1979 this effect was porposed as mentioned at the beginning of this apart. Other factor that need better understanding is the effect of ferhormones (as social clue), including steroids hormones, on the control of puberty onset; olfactory and vomeronasal organs are implicated but the exact pathways is not well defined. Finally, from a genetic improvement/reproductive management standpoint, is of interest the goal of shortening the time of onset of puberty, mainly in the male, in order to fasten the availability of spermatozoa production (particulary for artificial insemination in bulls, swine and poultry), the generation interval could be reduced and genetic improvement accelerated. Since female must maintain a successful pregnancy, deliver live offspring and lactate, there a clearly physiological limit to hastened puberty in females (Senger, 2006). The use of exogenous sex steroids for those purposes (male and female) is possible but also very questioned because of the secondary effects and the potential food residues (meat and milk) for human. Interestingly, Nelson proposed three potential predictors (i.e., biomarkers) of longevity in mammals (1) age of pubertal onset, (2) concentrations of gonadal steroids and (3) timing of age-related infertility. Ages of pubertal onset and of declining fertility are hypothesized to be positively correlated with longevity. Concentrations of androgens and estrogens are proposed to be inversely and positively correlated, respectively, with life span (Nelson, 1988).

### *3.4.2. Fertilization*

Thirty years ago research results about the effect of follicular steroids on the maturation and fertilization of mammalian oocytes was reported. Pronuclear development was used to measure the effects on ovine oocytes of altering follicular steroidogenesis during maturation *in vitro*. Follicular steroid secretion was altered using enzyme inhibitors and exogenous steroid supplementation. Abnormalities induced during maturation were measured 24 h after

transfer of oocytes to the oviducts of inseminated hosts. The authors concluded that oocytes require a specific intra-follicular steroid environment for the completion of the full maturation process. Alterations to the steroid profile during maturation induce changes in the oocyte which are expressed as gross abnormalities at fertilization (Moor et al., 1980).

Similarly, in other study, oocytes were collected by aspiration of preovulatory follicles from 55 women. After collection and culture, the oocytes were inseminated with the spermatozoa of the husband. The levels of progesterone, oestradiol-17β and androstenedione in the clear follicular fluid were measured by radioimmunoassay. A multivariate analysis containing these three hormone levels together with two ratios of progesterone with each of the other hormones indicated reasonable discrimination between the oocytes which fertilized and those which remained unfertilized after insemination. The discriminant analysis suggested that the fertilization of the oocytes could have been predicted on the basis of these hormonal profiles with a success rate which exceeded 90% (Fishel et al., 1983).

More recently, an academic article presents the result of a study on the correlation among sex steroids in follicular fluid (FF) and cultured granulosa cells and fertilization. The study examined the levels of E2, P4, and T in follicular fluid from stimulated cycles and their granulosa cell cultures after oocyte retrieval and the correlation between these levels. It revealed that there is no link among fertilization and sex steroid levels in FF and granulosa cells (FertilityWeekly, 2011). This is an important recent report taking in account that now a day in some in vitro fertilization –IVF- protocols, sexual steroids are commonly used as factor of fertilization improvement. Also, high follicular fluid E2 may be a marker for oocytes that will fertilize normally with intracytoplasmic sperm injection (ICSI) (Lamb et al., 2010).

At the spermatozoa level, in human it was demonstrated the expression of a functional surface estrogen receptor (of 29 KDa). Luconi et al., suggested that this receptor and of course its ligand, may play a role in the modulation of non-genomic action (via calcium modulation) of P4 in spermatozoa during the process of fertilization: E2 stimulates tyrosine phosphorylation of several sperm proteins, including the 29-KDa protein band, and determines a reduction of calcium response to P4, finally resulting in modulation of P4-stimulated sperm acrosome reaction in a dose-response manner (Luconi et al., 1999) (Table 2).

*3.4.3. Gestation and placentation*

The ontogeny and functional role of steroidogenesis during mammalian gestation is poorly understood. A 2002 review provides a summary of findings on the spatio-temporal expression of key steroidogenic genes controlling progesterone synthesis in the uterus during mouse pregnancy. Authors have shown that onset of P450scc and an identified isoform of murine 3beta-hydroxysteroid dehydrogenase/isomerase type VI (3betaHSD VI) expression occurs upon decidualization of the uterine wall induced by implantation. This unexpected early expression of the enzymes in the maternal decidua is terminated at mid-pregnancy when the steroidogenic ability reappears in the extraembryonic giant cells at the time of placentation. The giant cells express the StAR protein. Unlike the human placenta, the steroidogenic genes are not expressed in the cells of the mature mouse placenta during the second half of gestation. The results suggested that, during early phases of pregnancy, local P4 syn-

thesis in the maternal decidua and the trophoblast layers surrounding the embryonal cavity is important for successful implantation and/or maintenance of pregnancy. It was proposed that the local production of progesterone acts as an immunosuppressant at the materno fetal interface preventing the rejection of the fetal allograft (Ben-Zimra et al., 2002).

Strauss III et al. published in 1996 a review on the placental steroidogenesis capacity including the evidence for a dialogue between the ovary and the pituitary and placenta. In some mammals, the placenta eclipses the pituitary in the maintenance of ovarian function (e.g., mouse and rat). In human and in sheep, horse, cat, and guinea pig, the placenta acquires the ability to substitute for the ovaries in the maintenance of gestation at various times during pregnancy. They noted that even though the placentae of other species cannot substitute for ovarian function, all placentae critically studied expressed steroidogenic enzymes. Therefore, the ability to elaborate or metabolize steroid hormones is one common feature of trophoblast cells despite the marked differences in placental morphologies. In human, rhesus monkey, baboon, and horse, the placenta does not express 17a-hydroxylase. Placental estrogen synthesis in these species depends upon a source of androgen precursor from the fetus; the fetal adrenal glands in the case of primates, the gonadal interstitial cells in the case of the horse. In contrast, the trophoblast cells of rat, pig, sheep and cow express 17a-hydroxylase and are able to synthesize androgens and in some species estrogens.

In the rat, estrogen, synthesized by the ovaries, suppresses placental expression of 17a-hydroxylase. Since the rat placenta elaborates androgens that are potential precursors for ovarian aromatization, a dialogue between the placenta and ovary may take place in this species. Estrogens not only regulate 17a-hydroxylase expression, they control placental mass. The rat placenta hypertrophies in response to ovariectomy, and this hypertrophy is blocked by exogenous estrogen. These findings support the notion of an ovarian-placental interaction (Strauss et al., 1996) (Table2).

### *3.4.4. Parturition*

Since 1983, Meinecke-Tillnann et al. described the changes in the plasma levels of estrone and E2 during the estrous cycle, gestation and puerperium in the goat. Estrone sulphate and E2 concentrations rose until the 12th week of gestation and then declined to about 50% of the former ranges of concentrations before rising again to high values at weeks 17–20 of gestation. Increasing plasma levels of estrone sulphate and E2 were determined during the last ten days preceding parturition. The concentrations of estrone sulphate returned to basal levels by the $2^{nd}$-$4^{th}$ day post partum whereas oestradiol-17β values reached base values 24 hours after parturition. Both estrogen concentrations remained constant during the puerperium until day 51 post partum (Meinecke-Tillrnann et al., 1983). This complete described estrogene pattern is now a day well understood. In 2006, Senger clearly described the removal of "progesterone block" that occurs during mammals gestation and necessary to start parturition. Fetal cortisol promotes the synthesis of three enzymes that convert P4 to E2. Progesterone, that is high at the placenta interface (from gonadal or placental origin depending on the species, as explained before), is converted to 17 alpha-hydroxy-P4 by the enzyme 17alpha-hydroxylase. Fetal cortisol also induce the production of 17-20 desmolase to produce

androstenedione from the 17 alpha-hydroxy-P4 and then the induced enzyme aromatase converts androstenedione to estrogens; that is at the end a dramatic drop in P4 and a dramatic elevation in E2. The consecuences are that myometrium becomes increasingly more active and displays noticeable contractions. At the same time, fetal cortisol induces placental production of PGf2a which initiates the luteolytic process, contributing to the decrease of gonadal P4 production. Sex steroids and oxytocin (OT) produced within intrauterine tissues have been implicated in the regulation of parturition. Fang et al. performed very complete studies to determine the relationships among E2, P4, OT, and their receptors in uterine tissues during late gestation and parturition in the rat; to observe the effects of the estrogen antagonist tamoxifen (TAM) on these factors; and to evaluate the rat as a potential model for events at human parturition. Serum E2 increased throughout late gestation accompanied by an increase in uterine OT mRNA and ER. Serum P4 declined after day 19, and uterine PR did not change significantly. Uterine PGE2 increased progressively, reaching peak levels the evening before delivery. Uterine OTR did not increase until the morning of delivery, and uterine OT peptide concentrations increased only during parturition. Parturition was significantly delayed by 24 h in the TAM-treated group. TAM inhibited the increase in serum E2, uterine ER, and OT mRNA and peptide, but had no effect on serum P4 or uterine PR levels. With TAM, the responses of uterine OTR and prostaglandin E2 (PGE2) were significantly delayed, but still underwent a significant increase before the delayed parturition. These results supported that indeed E2 stimulates the synthesis of ER, OT, and OTR within the rat uterus and is essential for normal parturition. P4 withdrawal may be more important to the increases in OTR and PGE2, but these are delayed in the absence of estrogen (Fang et al., 1996). The precise temporal control of uterine contractility is essential for the success of pregnancy. For most of pregnancy, progesterone acting through genomic and non-genomic mechanisms promotes myometrial relaxation. At parturition the relaxatory actions of progesterone are nullified and the combined stimulatory actions of estrogens and other factors such as myometrial distention and immune/inflammatory cytokines, transform the myometrium to a highly contractile and excitable state leading to labor and delivery. Steroid hormone control myometrial contractility and parturition as part of the parturition cascade. (Mesiano and Welsh, 2007). The compulsory progesterone withdrawal necessary for deliveru take place is mediated by changes in myometrial expression of progesterone receptors (PRs)-a and –b. This withdrawal in human parturition may be mediated by an increase in the myometrial PR-a to PR-b ratio due to increased PR-a expression affecting myometrial cell progesterone responsiveness (Merlino et al., 2007) (Table2).

*3.4.5. Puerperium or postpartum*

In domestic animals, puerperium begins immediately after parturition and lasts until reproductive function in restored so that another ovulation occurs and other potential pregnancy can take place. The time required for complete uterine repair and ovarian activity to resume in the postpartum female varies significantly among species (beef cows: 30d and 50-60d; dairy cows: 45-50d and 25d; ewe: 30d and 180d; mare: 28d and 12 d; sow: 30d and 7d; queen: 30d and 30d; bitch: 90d and 150d, a long natural postpartum anestrus). In beef cow, sows and women, the lactation inhibits ovarian activity (Senger, 2006). Also, manipulation of ab-

normal anestrus in ruminants with sex steroids implants (P4,E2), intra muscular or intravaginal devices during postpartum are intended in order to shortening or at least to be near the normal period required to re-produce.

In beef cows (zebu-Bos indicus cattle), in some environmental conditions, the interval parturition-ovarian reactivation (anestrous period) and the abnormal sex steroids production represent a big economical problem (180-240 d, vs. 60d theorical proposed (Senger, 2006)) because animals are not producing during this large interval and the "physiological" goal of one calf a cow a year is not reached at all. This was investigated many years ago in the follicular morfological and steroids dynamics aspects concluding about very individual patterns and about the potential early capacity of initiating ovarian activity depending on many factors (Ruiz-Cortes and Olivera-Angel, 1999). The return to the ovarian activity postpartum, is determined by the recovery of the hipotalamic-hipofisis-ovary axis and mainly by three factors: (a) nutrition, by the secretion of leptin from adipocites, (b) suckling, by prolactin production and (c) the cow-calf link, mediated by the senses of the vision and smell. In addition, after ovarian recovery postpartum, the cows present low fertility associated with corpus luteum of short duration and low production of P4. The induction of estrus with progestins has generated corpus luteum of normal duration, in response to the weaning or to the injection of gonadotrophins. Zebu cows postpartum, were treated with progestins and with temporal suckling interruption (TSI):calves-cows separation, for 72 hours. We could conclude that the treatment with TSI solely or in combination with progestins, can induce estrus, ovulation and corpus luteum of good quality, in postpartum Zebu cows. This useful tool for shortennig calving intervals is now a day used with success by local farmers (Giraldo Echeverri et al., 2005).

Those features indicate mainly the multifactorial effects of the peripartum on the sex steroids production, but also the gonadal steroids important role in the pospartum cyclicity reactivation.

High levels of E2 near the delivery and some days after are also regulating the OTR expression and the OT and effects myometrium. Thus contractions needed for the placenta membranes and lochia (blood-tinged fluid containing remnants of the fetal placenta and endometrial tissue) discharge in the early postpartum occurs (Table 2).

Studies in primates have suggested that pre- and peripartum sex steroid hormones may be important determinants of maternal behavior and motivation, since higher levels of prepartum estrogen are associated with maternal competency and infant survivorship. The researchers found that high concentrations of prepartum $E_2$ in callitrichid primates are not necessarily associated with competent maternal behavior and may instead be associated with poor infant survivorship and inadequate maternal care. That appears to be convergent with research focusing on human mothers and may represent a common underlying mechanism linking prepartum estrogen and postpartum affect and behavior in some primates. Similary, in males of this specie, T, and possibly E2, play an important role in balancing the expression of paternal care with that of other reproductive behavior (Fite and French, 2000; Nunes et al., 2000).

### *3.4.6. Lactation*

The importance of the sex steroid hormones E2 and P4 for normal development of the mammary gland was recognized several decades ago and has been unequivocally confirmed since. This influence is not restricted to mammogenesis, but these hormones also control involution. Growth factors also have been shown to modulate survival (epidermal growth factor, amphiregulin, transforming growth factor $\alpha$, insulin like growth factor, and tumor necrosis factor $\alpha$) or apoptosis (tumor necrosis factor $\alpha$, transforming growth factor $\beta$) of mammary cells. Lamote et al. published in a review about the interaction between both groups of modulators as an important functional role for sex steroid hormones in the lactation cycle in co-operation with growth factors. At that time the molecular mechanism underlying the influence of sex steroid hormones and/or growth factors on the development and function of the mammary gland remained largely unknown (Lamote et al., 2004).

Nevertheless, in a model of *in vitro* mammary gland involution (mammary epithelial cells – MEC) where authors were interested in the autophagy and the apoptosis occurring during involution, they concluded about important molecular pathways explaining the sex steroids-growth factors cross-talk during lactation and involution. They investigated the effects of insulin-like growth factor-1 (IGF-I) and epidermal growth factor (EGF) signaling, as well as sex steroids on autophagy focusing about the role regulatory role of mTOR. The kinase mTOR links IGF-I and EGF signaling in inhibiting the autophagy pathways. Contrary to IGF-I and EGF, E2 and P4 exerted stimulatory effects on autophagy in bovine MEC. At the same time, it was a suppressive effect of both steroids on mTOR activation/phosphorylation. In conclusion, autophagy in bovine MEC undergoes complex regulation, where its activity is controlled by survival pathways dependent on IGF-I and EGF, which are involved in suppression of autophagy, and by pregnancy steroids, which act as inducers of the process (Sobolewska et al., 2009). Probably mammogenesis is also regulated by similar kinase pathway, and this is a clue finding to better understand sex regulation of mammalian lactation (Table 2).

Ovarian steroids (E2 and P4) diffuse directly from the blood into milk by passive diffusion because they are lipid soluble. All steroids hormones can be found in milk. The concentration of E2 and P4 in milk reflects cyclic hormone production by the ovaries and is highly correlated with blood concentrations. Such a phenomenon enables steroids (particulary P4) to be easely assayed in milk to determine the reproductive status of the female. In cows, the ELISA technology enables P4 levels in milk to be determined. The measurement of P4 in each milking through the use of "in-line" assay technology in the milking parlor is a revolutionary goal to achieve for research and for farmers producers management. The development of such technology would enable the producer to determine whether a cow is cycling, the stage of estrous cycle, pregnancy status and some form of ovarian pathology (v.g. cystic ovarian desease), for each cow, on a daily basis (Senger, 2006).

### *3.4.7. Menopause and andropause*

Menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity and marks the end of natural female reproductive life. Menopause

is preceded by a period of menstrual cycle irregularity, known as the menopause transition or peri-menopause, which usually begins in the mid-40s. The menopause transition is characterized by many hormonal changes predominantly caused by a marked decline in the ovarian follicle numbers. A significant decrease in inhibin B appears to be the first endocrine marker of the menopause transition with FSH levels being slightly raised. Marked decreases in estrogen and inhibin A with significant increases in FSH are only observed in the late stage of menopause transition. At the time of menopause, FSH levels have been shown to increase to 50% of final post-menopausal concentrations while estrogens levels have decreased to approximately 50% of the premenopausal concentrations. Since the decrease in estrogen levels occurs in the fifth decade of life, this means that most women will spend more than 30 years in postmenopausal status. A good body of evidence suggests that changes in hormonal status, particularly the decline in estrogen, in the menopause years may have a detrimental effect on women's health (Table 2). Accordingly, it has been reported that the decrease in estrogen contributes to the decrease in bone mass density, the redistribution of subcutaneous fat to the visceral area, the increased risk of cardiovascular disease and the decrease in quality of life.

In addition, hormonal changes may also have a direct effect on muscle mass. The measurement of urinary estrogens metabolites could add new evidence as for the role of estrogens in sarcopenia. It remains certain, though, that the decline in muscle mass is associated with an increased risk of functional impairment and physical disability. Finally, further randomized controlled trials are needed to investigate the effects of physical activity as well as hormone and phytoestrogen supplementation on sarcopenia (Messier et al., 2011).

Hot flushes common in almost 85% of women, appear to result from a dysfunction of thermoregulatory centers in the hypothalamus and are correlated with pulses of circulating estrogen and gonadotropin secretion in menopausal women (López et al., 2000).

A recent review of literature from 1990 until 2010, compare oral and transdermal delivery systems for postmenopausal estrogen therapy in domains of lipid effects; cardiovascular, inflammatory, and thrombotic effects; effect on insulin-like growth factor, insulin resistance, and metabolic syndrome; sexual effects; metabolic effects including weight; and effects on target organs bone, breast, and uterus.

Significant differences appear to exist between oral and transdermal estrogens in terms of hormonal bioavailability and metabolism, with implications for clinical efficacy, potential side effects, and risk profile of different hormone therapy options, but as neither results nor study designs were uniform, not complete conclusions could be done. Weight gain appears to be slightly lower with a transdermal delivery system. Oral estrogen's significant increase in hepatic sex hormone binding globulin production lowers testosterone availability compared with transdermal delivery, with clinically relevant effects on sexual vigor (Goodman, 2012).

The relationship between menopause and cognitive decline has been the subject of intense research since a number of studies have shown that hormone replacement therapy could reduce the risk of developing Alzheimer's disease (AD) in women. In contrast, research into andropause has only recently begun. Furthermore, evidence now suggests that steroidogenesis is not restricted to the gonads and adrenals, and that the brain is capable of producing

its own steroid hormones, including testosterone and estrogen (Bates et al., 2005). Male aging is associated with a variable but generally gradual decline in androgen activity, which can manifest as sexual dysfunction, lethargy, loss of muscle and bone mass, increased frailty, loss of balance, cognitive impairment and decreased general well-being, such as depression and irritability. Andropause is defined as the partial or relative deficiency of androgens and characteristic associated symptoms. These symptoms suggest that androgens may have an important modulatory role in cognition and mental health. Indeed memory loss was the third most common reported symptom of andropause, after erectile dysfunction and general weakness in a survey of elderly men (Bates et al., 2005).

Mild cognitive impairment (MCI) is becoming fashionable as a diagnosis, representing a state of cognitive decline associated with negligible functional loss. MCI is important as it often precedes Alzheimer disease (AD). Recognizing MCI may lead to preventive strategies that can delay the onset of AD. Many patients in transition into andropause report problems with their memory. There is strong evidence from basic sciences and epidemiological studies that both estrogens and androgens play a protective role in neurodegeneration. The evidence from small prospective clinical trials lends support to the role of hormones in improving cognitive function. Patients have reported memory improvements in both declarative and procedural domains after being on hormonal replacement. Authors have hypothesized androgens and perhaps selective androgen receptor modulators as future treatment options for MCI in aging males (Tan et al., 2003).

| **Moment** | **Definition*** | **Estradiol-E2 (effects, target tissues)** | **Progesterone-P4 (effects, target tissues)** | **Testosterone-T (effects, target tissues)** |
|---|---|---|---|---|
| Puberty | Acquisition of gonadotropin secretion, gametogenesis, gonadal steroids secretion, reproductive behaviour and secondary sex | Pituitary, Hypothalamus.<br>Increase responsiveness to GnRH<br>Increase of GH<br>Increase of IGF-I | | Genital enlargement<br>Muscle strength<br>Body composition<br>Spermatogenesis |
| Fertilization | The process of combining the male gamete, or sperm, with the female gamete, or ovum. The product of fertilization is a cell called a zygote | Oocytes.<br>Inhibits abnormalities<br>Stimulates maturation<br>Success of fertilization<br>Acrosome reaction | Oocytes.<br>Inhibits abnormalities<br>Stimulates maturation<br>Spermatozoa.<br>Acrosome reaction | Oocytes.<br>Inhibits abnormalities<br>Stimulates maturation<br>Success of fertilization |
| Gestation | Pregnancy. The period that a female is pregnant between conception and parturition | | Myometrium, decreases contractions<br>Endometrium, "maternal" secretions | |
| Placentation | The structural organization and physical relationship of the fetal membranes to the endometrium that | placenta<br>Control of placental mass<br>Inhibit 17a-hydroxylase expression in the placenta | Immunosuppressant of the placenta | Ovary. Cross talk with placental androgens |

| Moment | Definition* | Estradiol-E2 (effects, target tissues) | Progesterone-P4 (effects, target tissues) | Testosterone-T (effects, target tissues) |
|---|---|---|---|---|
| | provides the site of metabolic exchange between the dam and the fetus | | | |
| Parturition | To give birth | Ovary. Increases, OTR, production of PF2a<br>Endometrium. Increases lubrication<br>Myometrium. Increases ER, OTR, Increases contractions<br>Hypothalamus. Increases OT secretion | Ovary. Converted to E2 | |
| Postpartum or puerperium | The period between parturition and return to the normal cycling state of the ovaries and uterus | Brain. Male and female. Maternal and paternal behavior<br>Endometrium.<br>Myometrium.Contractions and placental membranes and loquia expulsion<br>Ovary: cross-talk with P4 | Ovary. Croos –talk with E2<br>Hypothalamus, Gn RH production control | Paternal behavior |
| Lactation | Formation and /or secretion of milk by the mammary glands | Mammary gland: development, mammogenesis<br>Cross-talk with IGF-I and EGF: modulation of lactation and involution (autophagy) | Mammary gland: development, mammogenesis<br>Cross-talk with IGF-I and EGF: modulation of lactation and involution (autophagy) | Mammary gland: development, mammogenesis<br>Cross-talk with IGF-I and EGF: modulation of lactation and involution (autophagy) |
| Menopause | Permanent cessation of menses; termination of menstrual cycles brought about by depletion of ovarian follicles | Bone. Regulates bone mass<br>Muscle. Regulates muscle mass<br>Subcutaneous visceral fat<br>Heart:cardiovascular desease<br>Brain: hot flushes, depression,irritability | | |
| Andropause | A variable complex of symptoms, including decreased Leydig cell numbers and androgen production, occurring in men after middle age | | | Bone. Mass loss<br>Muscle. Mass loss<br>Reprod. Tract.erectil dysfunction<br>Brain. Memory loss, cognitive impairment |

*modified from Senger, 2006

**Table 2.** Gonadal steroids regulation of clue reproductive moments. Definitions, target tissues and main sex steroids effects

## 4. Gonadal steroid hormones action on other systems

### 4.1. Energy homeostasis, sex hormones implications

Since the adipose tissue hormone leptin was discovered in 1996, its energy balance regulatory effects have been well investigated and accepted. The interaction of leptin and its membrane receptors within different systems were also the focus of interest of many researches making the protein and the receptor almost ubiquitous in mammals. Thus, it is of big interest the relationship of leptin with sex steroids. Early in this chapter, it was described how leptin regulates gonadal steroidogenesis (Montaño et al., 2009; Ruiz-Cortes et al., 2003; Ruiz-Cortes and Olivera, 2010). However, in 2000, Mystkowski and Schwartz postulated also that sex steroids and leptin regulate one another's production. Although gonadal steroids, unlike leptin, are clearly not critical to the maintenance of normal energy homeostasis, they do appear to function as physiologic modulators of this process. Gonadal steroids influence food intake and body weight. Although the specific mechanisms underlying these effects are not clear, a consideration of their effects in the context of current models of energy homeostasis may ultimately lead to the identification of these mechanisms. When compared with leptin, the prototypical humoral signal of energy balance, sex steroids share many common properties related to food intake and body weight. Specifically, gonadal steroids circulate in proportion to fat mass and current energy balance, and administration of these compounds influences food intake, energy expenditure, body weight, and body composition. Moreover, both estrogens and androgens modulate central nervous system effectors of energy homeostasis that are targets for the action of leptin, including pathways that contain neuropeptide Y, pro-opiomelanocortin, or melanin-concentrating hormone (Mystkowski and Schwartz, 2000).

Several studies have reported decreased circulating estradiol levels in type 1 and type 2 diabetic animal models. Women with type 1 diabetes experience decreased sexual arousal function and have significantly reduced E2 levels compared to control subjects. Limited data are available in type 2 diabetic women. It was proposed that diabetes disrupts estrogen signaling. This hypothesis was partially supported by studies showing that E2 supplementation in diabetic animals ameliorates some of the diabetic complications in several organs and tissues, including those that control anabolic and catabolic pathways (food intake and energy expenditure) such as melanocortin in the hypothalamic arcuate nucleus and neurons containing neuropeptide Y. No studies are available on the therapeutic effects of estradiol supplementation in type 2 diabetic animals in ameliorating the changes in sex steroid receptor expression and tissue localization and distribution. For these reasons, researchers undertook studies to investigate the effects of type 2 diabetes on the expression, localization and distribution of estrogen, androgen and P4 receptors and to determine if E2 treatment of diabetic animals normalizes these changes. They found decreased levels of plasma E2 and reduced ER expression in type 1 and type 2 diabetic animals suggesting that estrogen signaling is impaired in the diabetic state. They conclude specifically, in a vaginal model, that sex steroid hormone receptor signaling is important in female genital sexual arousal function. These

findings further demonstrate that E2 supplementation provides a protective effect by up-regulating the expression of sex steroid receptor proteins (Cushman et al., 2009).

Important tissues implicated in homeostasis are fat mass and muscle mass. Effects of androgens in those systems are well known. As general information, males typically have less body fat than females. Recent results indicate that androgens inhibit the ability of some fat cells to store lipids by blocking a signal transduction pathway that normally supports adipocyte function. Also, androgens, but not estrogens, increase beta adrenergic receptors while decreasing alpha adrenergic receptors resulting in increased levels of epinephrine/ norepinephrine due to lack of alpha-2 receptor negative feedback and decreased fat accumulation due to epinephrine/ norepinephrine then acting on lipolysis-inducing beta receptors.

About androgens and muscle mass, it is clear that males typically have more skeletal muscle mass than females and this is because androgens promote the enlargement of skeletal muscle cells and probably act in a coordinated manner to function by acting on several cell types in skeletal muscle tissue. One type of cell that conveys hormone signals to generating muscle is the myoblast. Higher androgen levels lead to increased expression of androgen receptor. Fusion of myoblasts generates myotubes, in a process that is linked to androgen receptor levels much more expressed in males but also having effect in females (Figure 6).

Sex hormones play essential roles in the regulation of appetite, eating behaviour and energy metabolism and have been implicated in several major clinical disorders in women. Estrogen inhibits food intake, whereas progesterone and testosterone may stimulate appetite. Interactions between sex hormones and neuroendocrinological mechanisms in the control of appetite and eating in women have been recently reviewed. Hirschberg indicates that the roles played by sex hormones in the development of eating disorders and obesity are clearer now a days. For instance, androgens may promote bulimia by stimulating appetite and reducing impulse control, a proposal supported by the observation that antiandrogenic treatment attenuates bulimic behaviour. Androgens are also involved in the pathophysiology of abdominal obesity in women. On the other hand, hormone replacement therapy with estrogen counteracts the weight gain and accumulation of abdominal fat associated with the menopausal transition. The author conclude that sex hormones and/or agents that exhibit similar activities may provide novel strategies for the treatment of eating disorders and android obesity, two of the most serious health problems for women today (Hirschberg, 2012).

### 4.2. Cardiovascular system

Sex steroids effects, as reviewed in the sex steroids molecular pathways section, have "the long" pathway and the rapid one. In the case of the cardiovascular system in mammals, the rapid non-transcriptional is the mechanism that explains the implications of gonadal steroids.

Traish et al. have recently reviewed the topic of androgens modulation of the lipid profiles and contribution to development and progression of atherosclerosis. They found studies in animals and humans suggesting that androgen deficiency is associated with increased triglycerides (TGs), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). Al-

though the effects of androgen deficiency on high-density lipoprotein cholesterol (HDL-C) remains controversial, recent data suggest that androgen therapy is associated with increased levels of HDL-C and may improve reverse cholesterol transport. Animal studies suggested that androgen deprivation adversely affect lipid profiles and this was reversed by androgen treatment. Furthermore, androgen treatment of hypogonadal men significantly improved lipid profiles. Emerging data indicate that androgens play an important role in lipid metabolism. Therefore androgens are critical in the prevention and progression of atherosclerosis (Traish et al., 2009a).

Androgen deficiency contributes to increased TGs, TC, LDL-C and reduced HDL-C while androgen treatment results in a favorable lipid profile, suggesting that androgens may provide a protective effect against the development and/or progression of atherosclerosis.

Until recently, it was thought that male gender contributes to the risk of atherosclerosis and this was attributed to androgens. Evidence is emerging that androgen deficiency is more likely to be associated with atherosclerosis than gender per se. T treatment of hypogonadal men resulted in reduced pro-inflammatory cytokines, total cholesterol, and triglyceride levels. In women,T use was reported for sexual dysfunction, abnormal uterine bleeding, dysmenorrhea, menopausal symptoms, chronic mastitis and lactation, and benign and malignant tumors of the breast, uterus, and ovaries (Traish et al., 2009b). However, these authors literally conclude: “health-care professionals engaged in the management of women's health issues have observed the benefits of androgen therapy throughout much of the 20th century. Despite this clinical use of testosterone in women for more than seven decades, contemporary testosterone therapy in women is hotly debated, misunderstood, and often misrepresented in the medical community”(Traish et al., 2009b).

New evidence suggests that androgen deficiency alters lipid profiles, which ultimately contribute to oxidative stress, endothelial dysfunction and increased production of pro-inflammatory factors, thus promoting the pathogenic process leading to atherosclerosis (Figure 6). Future research should focus on delineating the physiological or biochemical mechanisms and should focus on the molecular basis of androgen action in regulating lipid metabolism and endothelial function in order to have a better understanding of the role of androgens, deficiency and vascular diseases (Traish et al., 2009a).

### 4.3. Sex steroids, the brain and behavior

Almost all the sex steroids have something to do with the brain. It is maybe because of this part of the pathway that they are so important in the mammals general physiology (Figure 6).

Circulating levels of androgens can influence human behavior because some neurons are sensitive to steroid hormones. Androgen levels have been implicated in the regulation of human aggression and libido (Figure 6). Indeed, androgens are capable of altering the structure of the brain in several species, including mice, rats, and primates, producing sex differences. Numerous reports have outlined that androgens alone are capable of altering the

structure of the brain; however, it is difficult to identify which alterations in neuro-anatomy stem from androgens or estrogens, because of their potential for conversion.

Estrogens are effective regulators of brain cell morphology and tissue organization through the regulation of the cytoskeleton. Many of these regulatory actions related to cell morphology are achieved through rapid, non-classical signaling of sex steroid receptors to kinase cascades, independently from nuclear alteration of gene expression or protein synthesis. Brain cell morphology is then reported to be controlled by estrogens that regulate the development of neuron/neuron interconnections and dendritic spine density.This is thought to be critical for gender-specific differences in brain function and dysfunction. The recent advancements in the characterization of the molecular basis of the extra-nuclear signaling of estrogen helps to understand the role of estrogen in the brain and central nervous system, and may in the future turn out to be of relevance for clinical purposes (Sanchez and Simoncini, 2010).

Studies in animals have made abundantly clear the important role played by gonadal steroids in the regulation of behavior. Given the importance of reproductive behavior in the survival of the species, the potency and range (e.g., learning and memory, appetite, aggression, affiliation) of these behavioral effects are not surprising. The role of gonadal steroids in human behavior is both more complex and more poorly delineated.

The role of gonadal steroids in behavior in men and women include the exquisite context dependency of responses to gonadal steroid signals and the role of both gonadal steroids and context in several reproductive endocrine-related mood disorders such as menstrual cycle-related mood disorders, perimenopausal and periandropause depression, postpartum depression, hormone replacement therapy-related dysphoria, androgen-anabolic replacement, use or abuse(Rubinow et al., 2002).

Depression is more common in women, and women appear to respond better to selective serotonin reuptake inhibitors (SSRIs) than men. In addition, SSRIs are an excellent treatment for premenstrual dysphoria disorder. Thus, a sex specific effect of E2 and P4 on function of the serotonin transporter is quite important. However, the effect is the opposite of what would be predicted from the clinical literature, further underscoring the complexity of understanding the interactions between ovarian hormones and serotonin systems. Perhaps these findings help to better understand the vulnerability to mood disorders at times when E2 and P4 are high, such as the luteal phase of the menstrual cycle. Of note is the fact that these changes in response to E2 and P4 were not observed in hippocampi of male (Young and Becker, 2009).

In domestic animals the reproductive bevavior can take place only if the neurons in the hypothalamus have been sensitized to respond to sensory signals. T in the male is aromatized to E2 in the brain and E2 promotes reproductive behavior. In the male there is a relatively constant supply of T (every 4 to 6 h) and thus E2, to the hypothalamus. This allows the male to initiate reproductive behavior at any time. In contrast the female experiences high E2 during follicular phase only and will display sexual receptivity during estrus only. Under E2

influence, sensory inputs such as olfaction, audition, vision, and tactility send neural messages to the hypothalamus and cause the release of behavior specific peptides or neurotransmitters. In the mid brain, those hypothalamic signals are translated into fast responses. Synapsis between neurons of the mid brain and neurons in the medulla transmit the signal to the spinal cord and then to motor neurons that innervate muscles as during the lordosis and mounting occurring in domestic animals (Senger, 2006)

### 4.4. Gonadal steroids on bone turnover

In a very complete and recent review paper, Karsenty proposed that the well recognized sex steroid hormones regulation of bone mass accrual, is essential for skeletal development and maintenance of bone health throughout adult. Testosterone and estrogen positively influence growth, maturation, and maintenance of the female and male skeleton. Their effects are mediated mainly by slow genomic mechanisms through nuclear hormonal receptors, and possibly through the fast nongenomic mechanisms by membrane associated receptors and signaling cascades. But, on the other hand, the authors exposes the hypothesis that bone may regulates the female fertility by osteocalcin and that osteocalcin signaling in Leydig cells of the testis as a novel mode of regulation of testosterone synthesis observed in males but not in females (Karsenty, 2012 ).

Sex steroids play an important role in bone growth and the attainment of peak bone mass. They are, at least in part, responsible for the gender differences in bone growth, which emerges during adolescence. The skeletal sexual dimorphism is mainly due to a stimulatory androgen action on periosteal bone formation in men, whereas an inhibitory estrogen-related action occurs in women (Karsenty, 2012 ; Venken et al., 2006). In addition to the sex steroid hormones, several studies have shown that other hormones negatively regulated by estrogen, such as growth hormone (GH) and insulin-like growth factor 1 (IGF1), may further contribute to the development of the skeletal sexual dimorphism. Sex steroid hormones maintain skeletal integrity.

Testosterone and estrogens are also crucial for maintaining bone mass accrual during adulthood in the female and male skeleton. The loss of ovarian function underlies the development of osteoporosis (Karsenty, 2012 ; Vanderschueren et al., 2004). Estrogen deficiency is a major pathogenic factor in the bone loss associated with menopause and the development of osteoporosis in postmenopausal women. This rapid bone loss can be prevented by estrogen administration, and characteristically results in an increase in bone mineral density during the first months of treatment. Additionally, the loss of testicular function also underlies bone loss in men. Although osteoporosis more commonly affects women, the loss of androgens in males following castration or a decrease in androgen levels related to aging, during andropause, has the same dramatic effect on the skeleton. Androgens favor periostal bone formation in men, and maintain trabecular bone mass and integrity (Karsenty, 2012 ; Vanderschueren et al., 2004)(Figure 6).

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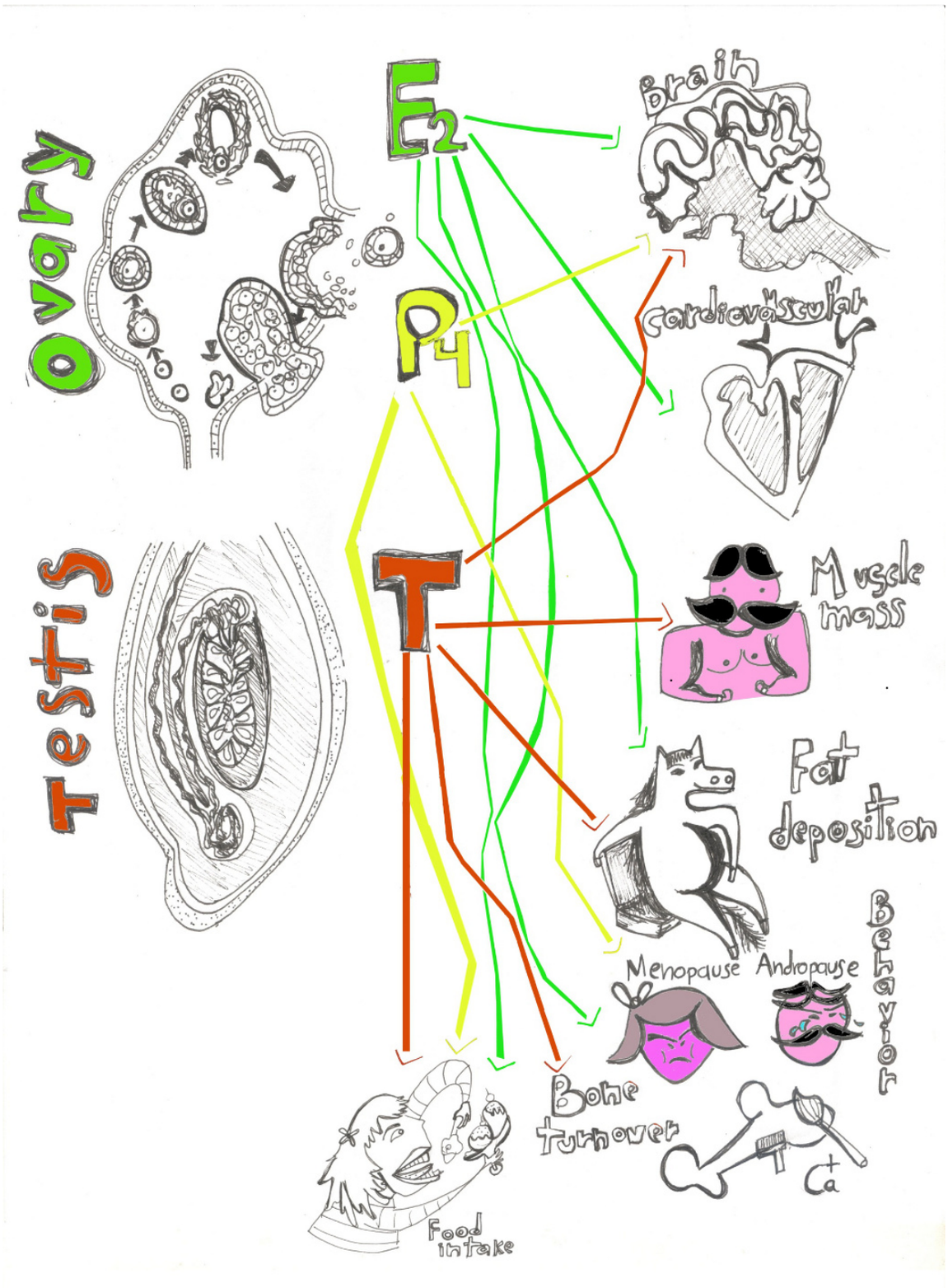


**Figure 6.** Sex steroids effects in different systems, a general view in mammals. Sex steroids produced in ovary and testis are regulating different organs and tissues in the brain (behavior,menopause,andropause), food intake and general homeostasis, cardiovascular system, muscle, fat and bone mass.

## 5. Conclusions and perspectives

Paraphrasing a paper first sentence: "Sex matters to every cell of the body" (Young and Becker, 2009) and gonadal steroids are mediating such a huge task. Since the discovery of those hormones (eighteen century), androgens, estrogens and progestogens, were classified more in a sex manner. It is well known now that males and females share the steroidogenesis pathways and sex steroids effects in many organs and systems such as the brain, muscle, fat, bone, reproductive system, cardiovascular system, homeostasis system (body weight-composition, food intake). The unraveling of this complex panorama of action and interaction of gonadal sex steroids indicates that almost no organ is left out of the sex hormones scope. One the most interesting feature of sex steroids action are the alternates pathways they are using to exert they modulating role by their membrane, cytoplasmic or nuclear receptor molecular activation (rapid and/or genomic mechanism) or in the very pertinent called "cross-talk" with other hormones, growth factors, transcription factors signaling pathways. Organism are integrated entities fulfilling their specific functions, and not as isolated group of distinct cell types (Karsenty, 2012 ) and it is amazing to realize how gonads via sex steroids are able to modulate and integrate such an intrincate orchestra. From a molecular point of view, the multiple signaling pathways already described for androgens and estrogens and their interactions are just the beginning of the understanding of the complex cross talk and interorgan connections. Some species can support gestation with just gonadal-corpus luteum P4 synthesis; other early during pregnancy can afford ovariectomy and the placenta would replace the steroid production. This turnover do not occur as an isolated event but more as unique interaction or cross-talk between placenta and ovaries (placental androgens aromatized in the ovary); similar interaction is happening between ovarian theca and granulosa cells and between Leydig and Sertoli testicular cells where androgens synthesis and aromatization to estradiol take place in a paracrine manner. Other amazing phenomenum is the autocrine dialogue in granulosa cells during luteinization, where granulosa producing high concentration of E2 switch their steroid production to P4 secretion. Again in this case the molecular machinery implicated in the sex steroid production is model of versatility and adaptation. Thus, the evolutionary aspect should be taken also in account for future studies about the cellular and molecular pathways of gonadal sex steroids action and interaction in mammals.

There are still several pieces of the puzzle that are to be found. Perspective to further research includes studies on the new class of steroid receptors (implicated in the rapid action signaling); the exact role of E2 and inhibin in male reproduction; the anabolic synthetic products:use and abuse; the therapy of replacement in menopause and andropause: what is worst the loss of sex steroids, or the "non-natural" replacement therapy with cancer risk? What is the matter, the delivery system, the doses, the compounds (what about phytotherapy?), the duration of the treatment, the human being individuality and conscience? In domestic animals, sex steroids therapies, to regulate cyclicity or even to make monovulatory animals to superovulate, are very common practice and collateral effects are rare or inexistent. Of course, they are not long treatment but instead they are performed very frequently with remarkable positive results. The ultimate purpose of this review was not to propose the

ideal steroid therapy or to give all the molecular factors implicated in the action and interaction of sex steroids in mammals. However, it could allow a better understanding of the panoply of effects in reproductive and other systems.

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