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# Sex Hormones and Infertility

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## 1. Introduction

The normal physiology of the female reproductive system involves a hypothalamus that secretes gonadotropin-releasing hormone (GnRH)in a pulsatile manner, a pituitary gland that can be stimulated by the hypothalamus to regularly secrete both luteinizing hormone (LH) and follicle-stimulating hormone (FSH), an ovary that has both methodical enzymatic system and steroidogenesis for producing the sex hormones such as estrogen and progesteron, and a functional uterus that can be responded by these hormones.
Sex hormones play a crucial role in reproductive biology as well as in general physiology. The most important aim of sex hormones is to design the cycle and to produce an optimal environment for pregnancy according to form ovarian physiology including follicular growth, ovulation, and corpus luteum formation and endometrial response including proliferative and secretuar phase for implantation. Among the various functions, sex hormones influence pregnancy, cardiovascular function, bone metabolism, and an individual's sense of general well-being. The action of sex hormones is mediated via extracellular signals to the nucleus to affect a physiologic response.

## 2. Gonadotropin-releasing hormone (GnRH)

Gonadotropin-releasing hormone (GnRH) is a decapeptide pulsatile produced by neurons with cell bodies primarily in the arcuate nucleus of the hypothalamus (1). Embryologically, these neurons originate from the olfactory area and then migrate to their adult locations (2). These GnRH-secreting neurons project axons that terminate on the portal vessels at the median eminence where GnRH is secreted for delivery to the anterior pituitary. The continual pulsatile secretion of GnRH is necessary because its short half-life is only 2–4 minutes as a result of rapid proteolytic cleavage.
GnRH stimulates the production, secretion and storage of FSH and LH from anterior hypophysis. (3). It is also an unique releasing hormone for the regulation of the simultaneous secretion of two hormones in human body (4). GnRH performs this special affect according to its pulsatile secretion. In the follicular phase, its secretion is characterized by frequent, small-amplitude pulses, however during the luteal phase, there is a progressive lengthening of the interval between pulses with higher amplitude (5).
GnRH is primarily involved in endocrine regulation of gonadotropin secretion from the pituitary. However, the regulation of GnRH secretion is various (Table 1). The pulsatile secretion of GnRH is directly affected by catecholaminergic system including the activator of

| Inhibitors of GnRH secretion | Activators of GnRH secretion |
|---|---|
| Dopamine | Norepinephrin |
| Gonadotrophins (negative feedback) | Catecholamins |
| Endogenous opioids | Neuropeptide Y |
| Estradiol | Acetylcholine |
| Progesterone | VIP |
| CRH | Naloxone |
| Melatonin | |
| Serotonin | |
| GABA | |

Table 1. The control of GnRH secretion

norepinephrine and the inhibitor of dopamin. This system is basically regulated by endogenous opioids (6).

These opioids are three groups;

1. Endorphins
2. Enkephalins
3. Dynorphins

Endogenous opioids, inhibit the gonadotropin secretion to swage the GnRH secretion from hypothalamus.

Sex steroids affect GnRH by increasing the secretion of the endogenous opioids in the central nervous system (7).

Although estrogen stimulates the secretion of endogenous opioids, estrogen plus progesterone increase this effect. Clinically, increased endogenous opioids may cause hypothalamic amenorrhea.

## 3. GnRH analogs

### 3.1 GnRH agonists (leuprolide, goserelin, nafarelin, buserelin)

GnRH agonists are modifications of the native molecule to either increase receptor affinity or decrease degradation (8). The pulsatile usage of GnRH agonists that resemble endogenous GnRH leads to increase the secretion of FSH and LH. However the constant GnRH usage leads to suppression of gonadotropin secretion by the downregulation of its receptor. An initial release of gonadotropins is followed by a profound suppression of secretion. The initial release of gonadotropins represents the secretion of pituitary stores in response to receptor binding and activation. With continued activation of the gonadotroph GnRH receptor, however, there is a downregulation effect and a decrease in the concentration of GnRH receptors. As a result, gonadotropin secretion decreases and sex steroid production falls to castrate levels (9).

The most commonly used regimen for superovulation in ART is called the long, or luteal, downregulation protocol. In this protocol, GnRH agonist is started in the luteal phase (day 21) of the previous cycle, which minimizes its flare effect and prevents the follicular recruitment that is thought to begin in the luteal phase. The couple undergoing treatment is advised to abstain from intercourse during the cycle before the start of COH; however, concomitant use of GnRH agonist in the presence of an unsuspected pregnancy has not been reported to be associated with increased spontaneous abortion, congenital abnormalities, or pregnancy

complications. Most importantly, clinical pregnancy rates and live birth rates per retrieval were significantly higher using the long protocol. The benefits including higher pregnancy rates and lower OHSS rates of using the long protocol for administration of GnRH agonists greatly outweigh its disadvantages, which include daily administration, increased requirement for gonadotropins, and an overall increase in the cost of medication (10, 11).

### 3.2 GnRH antagonists (cetrorelix, ganirelix)

GnRH antagonists produce a competitive blockage of GnRH receptors, preventing stimulation by endogenous GnRH and causing an immediate fall in gonadotropin and sex steroid secretion. The clinical effect is generally observed within 24 to 72 hours. Moreover, antagonists may not show flare-up affect comparing with GnRH agonists. GnRH antagonists are also used in ART for the prevention of premature ovulation and displays similar efficacy comparing GnRH agonist (long protocol) (12). However, there were significantly fewer pregnancies with the GnRH antagonist protocol. A significant reduction in the incidence of severe OHSS and the number of gonadotropin injections were observed in the antagonist regimen compared with the long GnRH–agonist protocol.

When we searched the Cochrane Library and ACOG Commitee on Practice Bulletins.;

Clinically usage of GnRH analogs listed below;

- Endometriosis
- Hormon dependent neoplasia such as endometrium cancer, breast cancer.
- Myomas of uterus
- Precocious puberty
- Dysfunctional uterine bleeding
- Ovarian hyperandrogenism
- Premenstrual syndrome
- ART for control of premature ovulation

Side effects of GnRH analogs listed below;

- Hypoestrogenic state
- Vasomotor symptoms
- Vaginal dryness
- Mood changes
- Loss of bone mineral density

Usage of these agents is generally limited to 6 months because of the adverse effects as listed above. The most important side effect is osteoporosis. Many side effects can be minimized by providing add-back therapy in addition to the agents.The addition of 2.5 mg of norethindrone or 0.625 mg of conjugated estrogens with 5 mg/d of medroxyprogesterone acetate seems to relieve these side effects of GnRH analogs. The addition of 5 mg daily of norethindrone acetate alone or in conjunction with low-dose conjugated equine estrogen seems to eliminate the loss of bone mineral density effectively as well (13).

## 4. Gonadotropins

The gonadotropins FSH and LH are produced by the anterior pituitary gonadotroph cells and are responsible for ovarian follicular stimulation. Structurally, there is great similarity between FSH and LH. They are both glycoproteins that share identical α subunits and differ only in the structure of their β subunits, which confer receptor specificity (14). The synthesis

of the β subunits is the rate-regulating step in gonadotropin biosynthesis (Lalloz MRA, et al. GnRH desensitization preferentially inhibits expression of the LH β-subunit gene in vivo. Endocrinology 1988;122:1689–1694.). Thyroid-stimulating hormone and placental human chorionic gonadotropin (hCG) also share identical α subunits with the gonadotropins. The structural similarity between FSH, LH, TSH and hCG defines as the α subunits identical and the β subunits differ.

The gonadotropins were metabolized in liver and kidney then excreted by the way of urine. The half life of LH, FSH and hCG is 20 minute, 3-4 hours and 24-36 hours, respectively.

### 4.1 FSH

Receptors of FSH are found on granulosa cells.

FSH plays role in; granulosa cell proliferation in follicules and estrogen production

- the production of FSH and LH receptors on granulosa cells
- the activation of aromatase and 3 beta-hydroxysteroid dehydogenase
- enzymes
- the stimulation of follicules and prevention of apoptosis of them

In the beginning of follicular development, there is no LH receptor on granulosa cells, however, during the 11-12nd days of cycle, FSH stimulates the production of LH receptors on granulosa cells.

### 4.2 LH

Receptors of LH are found on theca cells.

LH plays role in; internal thecal cell proliferation in follicules and androgen production

- luteinization and the production of progesterone when LH receptors found on
- granulosa cells during the 11-12nd days of cycle
- providing of ovulation
- the completion of I. myosis (the transformation of primary oocyte to
- secondary oocyte)

Although FSH plays an important role for the early maturation of follicules, FSH and LH are responsible together for the maturation of follicules before the ovulation.

## 5. Inhibin

Inhibin secretes from granulosa cells, sertoli cells, placenta and the basophilic cells of hypophysis. In the cycle, Inhibin selectively inhibits the secretion of FSH. There are two forms; Inhibin A and Inhibin B. The affect of Inhibin B mostly shows on follicular phase, but the affect of Inhibin A mostly shows on luteal phase of cycle. During luteofollicular transition of cycle FSH increase by decreasing of Inhibin A levels. Inhibin also stimulates LH activity and IGF secretion from granulosa and theca cells to increase androgen production.

## 6. Activin

Activin secretes from granulosa cells and the basophilic cells of hypophysis. In the cycle, Activin selectively activates the secretion of FSH. Therefore, it activates all affects of FSH on granulosa cells. Activin also inhibits LH activity, androgen production from theca cells and progesterone production from granulosa cells. Additionally, activin inhibits the secretion of IGF from ovary and the secretion of prolactin, ACTH, and GH from hypophysis. These

affects of activin is inhibited by inhibin and follistatin. Follistatin that secretes from granulosa cells and the basophilic cells of hypophysis inhibits FSH activity by binding activin.

### 6.1 IGF

IGF secretes from granulosa cells and theca cells. Its affect is similar to GH.
IGF plays role in; the stimulation of LH activity on theca cells to increase androgen production

- the production of FSH and LH receptors on granulosa cells
- the activation of aromatase enzyme
- the proliferation of granulosa cells
- the improvement of progesterone synthesis

All of IGF binds insulin like growth factor binding protein (IGF-BP) in the circulation. FSH and insulin inhibit the synthesis of IGF-BP, so efficacy of IGF may increase by increasing free IGF.
Epidermal growth factor (EGF) that is an important inhibitor of FSH in the ovary is another growth factor.

## 7. Steroid hormones in reproduction

Sex steroid hormones are synthesized in the gonads, adrenal gland, and placenta. Cholesterol is the primary building block in steroidogenesis, and all steroid-producing tissues except the placenta are capable of synthesizing cholesterol from the 2-carbon precursor, acetate. Steroid hormone production, which involves at least 17 enzymes, primarily occurs in the abundant smooth endoplasmic reticulum found in steroidogenic cells.
Steroids are metabolized mainly in the liver and to a lesser extent in the kidney and intestinal mucosa. Accordingly, administration of certain pharmacologic steroid hormones may be contraindicated in those with active liver disease. Sex steroids are divided into three groups based on the number of carbon atoms that they contain. Each carbon in this structure is assigned a number identifier, and each ring is assigned a letter. The 21-carbon series includes progestins as well as glucocorticoids and mineralocorticoids. Androgens contain 19 carbons, whereas estrogens have 18. However the ovary is deficient in 21-hydroxylase and 11 -hydroxylase and therefore is unable to produce corticosteroids. Most important steroidogenic enzymes are listed in Table 2. Steroidogenesis is summarized in Figure 1.

| **Enzyme Cellular** | **Location** | **Reactions** |
|---|---|---|
| P450scc | Mitochondria | Cholesterol side chain cleavage |
| P450c11 | Mitochondria | 11-Hydroxylase<br>18-Hydroxylase<br>19-Methyloxidase |
| P450c17 | Endoplasmic reticulum | 17-Hydroxylase<br>17, 20-Lyase |
| P450c21 | Endoplasmic reticulum | 21-Hydroxylase |
| P450arom | Endoplasmic reticulum | Aromatase |

Table 2. Steroidogenic enzymes

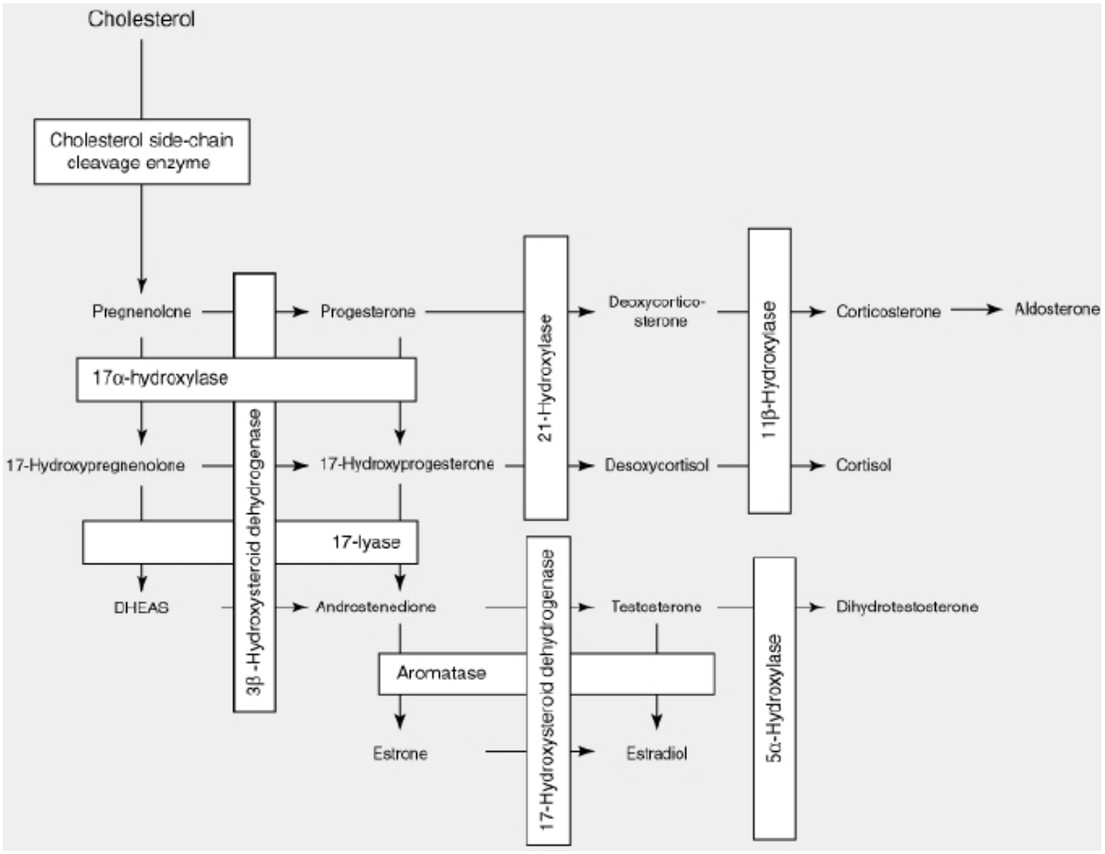



Fig. 1. The steps of the steroidogenesis pathway

Most steroids in the peripheral circulation are bound to carrier proteins, either specific proteins such as sex-hormone binding globulin (SHBG) or corticosteroid-binding globulin, or to nonspecific proteins such as albumin. Only 1 to 2 percent of androgens and estrogens are unbound or free. Tablo 3 shows the steroid transformations. Levels of SHBG are increased by hyperthyroidism, pregnancy, and estrogen administration. In contrast, androgens, progestins, GH, insulin, and corticoids decrease SHBG levels.

## 8. Estrogens

Steroids with 18 C classified as;
Estron (E1): Poor estrogenic affect, basically peripheric estrogen are the properties. It is dominant estrogen in prepubertal and postmenopausal periods.
Estradiol (E2): The most potent estrogen is mainly produced in the reproductive age.
Estratriol (E3): The least potent estrogen is mainly produced in pregnancy and synthesized by maternal and fetal units together. Therefore, E3 is an indicator to show the normal fetoplacental unit.
Estetrol (E4): There is not estrogenic affect. It is synthesized from fetal liver and increases in term.
Estrogens are produces by the aromatization of androstenedione and testosterone in ovary and the peripheral aromatization of androstenedione in skin, fat tissue, muscle and endometrium (Figure 2). There is a two-cell theory of ovarian steroidogenesis. The two-cell

theory of ovarian steroidogenesis explains that estrogen biosynthesis requires the combined action of two gonadotropins (LH and FSH) on two cell types (theca and granulosa cells) (15). Until the late antral stage of follicular development, LH-receptor expression is limited to the thecal compartment and FSH-receptor expression is limited to the granulosa cells. Theca cells express all of the genes needed to produce androstenedione. This includes high levels of CYP17 gene expression, whose enzyme product catalyzes 17-hydroxylationation the rate-limiting step in the conversion of progesterones to androgens (16). This enzyme is absent in the granulosa cells, so they are incapable of producing the precursor needed to produce estrogens by themselves. Granulosa cells therefore rely on the theca cells as their primary source for estrogen precursors. In response to LH stimulation, theca cells synthesize the androgens, androstenedione and testosterone. These androgens are secreted into the extracellular fluid and diffuse across the basement membrane to the granulosa cells to provide precursors for estrogen production. In contrast to theca cells, granulosa cells express high levels of aromatase activity in response to FSH stimulation. Thus, these cells efficiently convert androgens to estrogens, primarily the potent estrogen, estradiol. In sum, ovarian steroidogenesis is dependent on the effects of LH and FSH acting independently on the theca cells and granulosa cells, respectively.

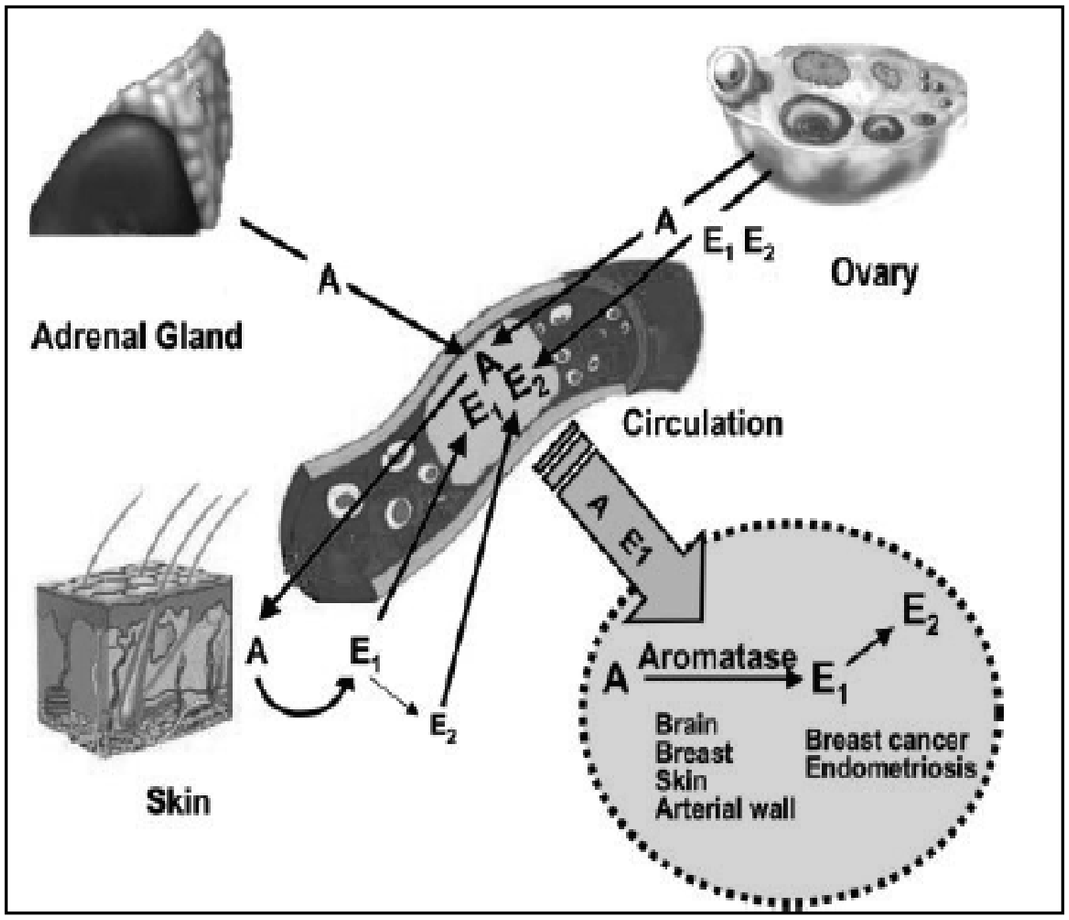



Fig. 2. Estrogen biosynthesis

Estrogen is metabolized in liver and excreted to bile.

The effects of estrogen on;
Genitourinary system:
- Stimulation of urethral epithelial proliferation
- Stimulation of vaginal epithelial proliferation and superficial cells of epithelium may become dominant in vagina.
- Decreases the vaginal pH (3.8-4.5)
- Increases the cervical mucus and elasticity and decreases the cervical viscosity (Spinn-Barkeit)
- Increases the crystallization of NaCl in cervical mucus and may cause ferning image.
- Increase cervical mucus pH
- Proliferation of endometrial stroma and glands
- Production of endometrial progesterone receptor
- Increase the gap junctions, connexion proteins, and oxytocin sensitivity of smooth muscles in uterus. Therefore, estrogen increases the uterin contractility.
- Increase the ciliary activity and motility in fallopian tubes
- Facilitate follicular stimulation
- Inhibits FSH (negative feed-back effect)
- Positive feed-back on LH before the ovulation
- Inhibits GnRH (Increase central opioids)

Breast
- Development of ductus

Seconder sex characters
- Development of axillary and pubic hairs in puberty (pubarche)
- Development of breast in puberty (thelarche)

Bone
- Increase the osteoblastic activity in bone and bone mineral density

Skin
- Increase vascularity and collagen

Liver
- Increase SHBG synthesis
- Increase transcortin synthesis
- Increase angiotensinogen
- Increase coagulation factors as Factor II, VII, VIII, IX, and X
- Decrease antithrombin production
- Increase triglycerides, total cholesterol, and HDL
- Decrease LDL
- Increase concentration of bile acids and the development of cholelithiasis

Estrogens exert a variety of effects on growth and development of different tissues. The effects of estrogens are mediated via estrogen receptors (ER), intracellular proteins that function as ligand-activated transcription factors and belong to the nuclear receptor superfamily. Two mammalian ERs have been identified, denoted ERα and ERβ. The structure of both receptors is similar and consists of six domains named A through F from the N- to C-terminus, encoded by 8 to 9 exons. Genes that are regulated by activated ERs include early gene responses such as c-myc, c-fos, and d-jun, as well as genes encoding for growth factors such as insulin growth factor (IGF-1 and IGF-2), epidermal growth factor (EGF), transforming growth factor-α, and colony-stimulating factor (CSF-1).

In addition to the described genomic effects of estrogens, there is growing evidence for nongenomic effects of estrogens on intracellular signal transduction pathways. These effects

include, for example, rapid activation of the adenylate cyclase, which results in cyclic adenosine monophosphate (cAMP)–dependent activation of protein kinase A (PKA). Estrogens can also stimulate the mitogen-activated protein kinase (MAPK) pathways and rapidly activate the Erk1/Erk2 proteins (17).

| | Free | Albumin | SHBG | Transcortin |
|---|---|---|---|---|
| Estrogen | % 1 | % 30 | % 69 | - |
| Testosterone | % 1-2 | % 20-32 | % 66-78 | - |
| DHEA | % 4 | % 88 | % 8 | - |
| Androstenedione | % 7 | % 85 | % 8 | - |
| DHT | % 1 | % 71 | % 28 | - |
| Progesterone | % 2 | % 80 | % 1> | % 18 |
| Cortisol | % 10 | % 15 | - | % 75 |

Table 3. Steroid transformations

The combined production of estradiol and inhibin B by the dominant follicle results in the decline of follicular phase FSH levels, and at least in part, may be responsible for the failure of other follicles to reach preovulatory status during any one cycle. This model predicts that follicles that lack adequate FSH receptor and granulosa cell number will remain primarily androgenic and will therefore become atretic. In support of this model, an increased androgen:estrogen ratio is found in the follicular fluid of atretic follicles and a number of studies have demonstrated that high estrogen levels prevent apoptosis. IGF also has apoptosis-suppressing activity, and is produced by granulosa cells. This action of IGF-I is suppressed by certain IGF-binding proteins that are present in the follicular fluid of atretic follicles. The action of FSH to prevent atresia may therefore result, in part, from its ability to stimulate IGF-I synthesis and suppress the synthesis of the IGF-binding proteins.
Clinically, there are some selective estrogen receptor modulators (SERM) such as clomiphene citrate (CC), tamoxifen, and raloxifen (Table 4).

| | Breast | Genital | Kemik | Lipid |
|---|---|---|---|---|
| *Clomiphene citrate* | + | _ | + | + |
| *Tamoxifen,* | _ | + | + | + |
| *Raloxifen* | _ | _ | + | + |

Table 4. The effects of selective estrogen receptor modulators on some tissues

CC is the initial treatment for most anovulatory infertile women. Chemically similar to tamoxifen, CC is a nonsteroidal triphenylethylene derivative that demonstrates both estrogen agonist and antagonist properties. Antagonist properties predominate except at very low estrogen levels. As a result, negative feedback that is normally produced by estrogen in the hypothalamus is reduced. Gonadotropin-releasing hormone (GnRH) secretion is improved and stimulates pituitary gonadotropin release. The resulting increase in follicle-stimulating hormone (FSH), in turn, drives ovarian follicular activity.

The SERM tamoxifen is an estrogen antagonist in the breast that is used in the treatment of estrogen-receptor positive breast cancer. Tamoxifen (20 mg) also has been approved for the prevention of breast cancer in high-risk women, resulting in an approximately 50% reduction in the risk of disease (18).
Raloxifene is a SERM that has been approved for both the prevention and treatment of osteoporosis. Raloxifene exercises estrogen-like actions on bone and lipids without stimulating the breast or endometrium. Raloxifene also may reduce the risk of breast cancer. Postmenopausal women receiving raloxifene as part of a large osteoporosis treatment trial experienced a 76% reduction in the risk of invasive breast cancer compared with placebo-treated women (19).

| Androgens | Potence | Ovary | Adrenal | Peripheral |
|---|---|---|---|---|
| DHEA | _ | % 25 | % 50 | % 25 |
| DHEAS | 5 | _ | % 100 | _ |
| Androstenedione | 10 | % 50 | % 50 | _ |
| Testosterone | 100 | % 25 | % 25 | % 50 |
| DHT | 300 | _ | _ | % 100 |

Table 5. Androgen biosynthesis

## 9. Progesterone

Progesterone is the 21 C steroid that secretes mainly from corpus luteum and placenta. It minimally secretes from the cortex of adrenal gland. Although its level is 1 ng/mL in preovulatuary phase, it is 3-15 ng/mL in luteal phase. Also, progesterone has a thermogenic affect.
The effects of progesterone on;
Genitourinary system:

- Intermediate cells of epithelium may become dominant in vagina.
- Increases the vaginal pH (> 4.5)
- Increases the cervical viscosity and decreases the cervical mucus and elasticity
- Decreases cervical mucus pH
- Antiproliferative and antimitotic effects on endometrial stroma and glands
- Secretuar changes on endometrium for implantation
- Decraeses the gap junctions, connexion proteins, and oxytocin sensitivity of smooth muscles in uterus. Therefore, progesterone decreases the uterin contractility
- Decrease the ciliary activity and motility in fallopian tubes
- Antiestrogenic activity according to the decrease in estrogen receptor and the increase in transformation of E2 to E1 (stimulates 17 OHSD enzyme)
- Inhibits LH (negative feed-back effect)
- Positive feed-back on FSH before the ovulation
- Inhibits GnRH (Increase central opioids)

Breast

- Development of alveols and lobules

Bone

- Antiresorptive effects on bone and increase bone mineral density

Liver

- Decrease SHBG synthesis

Most progesterone actions on the female reproductive tract are mediated through nuclear hormone receptors. Progesterone enters cells by diffusion and in responsive tissues becomes associated with progesterone receptors (Conneely OM, et al: Reproductive functions of progesterone receptors. Recent Prog Horm Res 57:339, 2002). There are multiple isoforms of the human progesterone receptor. The best understood isoforms are the progesterone receptor type A (PR-A) and B (PR-B). Both arise from a single gene, are members of the steroid receptor superfamily of transcription factors, and regulate transcription of target genes. These receptors have unique actions. When PR-A and PR-B receptors are co-expressed, it appears that PR-A can inhibit PR-B gene regulation. The inhibitory effect of PR-A may extend to actions on other steroid receptors, including estrogen receptors.

## 10. Androgens

The ovary produces primarily androstenedione and dehydroepiandrosterone (DHEA) with small amounts of testosterone. Although the adrenal cortex primarily produces mineralocorticoids and glucocorticoids, it also contributes to approximately one-half of the daily production of androstenedione, DHEA, and essentially all of the sulfated form of DHEA (DHEAS). Twenty-five percent of circulating testosterone is secreted by the ovary, 25 percent is secreted by the adrenal gland, and the remaining 50 percent is produced by peripheral conversion of androstenedione to testosterone (Figure 3).

## 11. Anti-Müllerian hormone (AMH)

AMH has been identified as a dimeric glycoprotein and a member of the transforming growth factor beta (TGFb) family of growth and differentiation factors. The pool of primordial follicles in the ovary is related to the number of growing antral follicles. Antral follicles are responsive to gonadotrophin stimulation and the measure of ovarian reserve can be defined as the total number of follicles, which can be stimulated to grow under maximal stimulation. Classically, age, FSH levels in the early follicular phase, antral follicle count and inhibin B have been used as markers of ovarian reserve. More recently, AMH, have been used by various groups to assess the ovarian reserve (20).

AMH is initially expressed in ovarian granulosa cells of primary follicles, maximal expression occurs in pre-antral and small antral follicles. Antral follicles measuring <6 mm express the greatest amount of AMH, and that expression declines as antral follicles increase in size. AMH is not expressed by atretic follicles and during FSH dependent final stages of follicular growth. AMH inhibits initial primordial follicles recruitment and decreases the sensitivity of preantral and small antral follicles to FSH. Serum AMH concentrations decline with increasing age and constitute a sensitive marker for ovarian aging. Recently, AMH is used as pretreatment assessment of ovarian reserve.

Basal serum levels of AMH may more accurately reflect the total developing follicular cohort and consequently potential ovarian response to FSH in cycles of ART. AMH, antral follicle count, inhibin B, FSH and ovarian volume have been demonstrated to reflect ovarian reserve.

## 12. Infertility

Infertility is defined as 1 year of unprotected intercourse without pregnancy. This condition may be further classified as primary infertility, in which no previous pregnancies have occurred, and secondary infertility, in which a prior pregnancy, although not necessarily a live birth, has occurred. Infertility affects about 10% to 15% of reproductive age couples. (21). Various factors may be responsible for the inability to achieve a successful pregnancy. Ovulatory, anatomic, immunologic, or hormonal factors on the woman's side and abnormalities of the semen parameters on the man's side are the most common (Table 6). After a thorough work-up, treatment can be planned that aims to correct the problem identified or, in the case of unexplained infertility, tries to improve all steps of the reproductive process.

| | Prevalence of the etiologies of infertility (%) |
|---|---|
| Male factor | 25–40 |
| Female factor<br>- Ovulatory dysfunction<br>- Tubal or periotoneal factor<br>- Unexplained infertility<br>- Miscellaneous causes | 40–55<br>30–40<br>30–40<br>10–15<br>10–15 |
| Both male and female factors | 10 |
| Unexplained infertility | 10 |

Table 6. Causes of Infertility

## 13. Evaluation of infertility

The most important tests for evaluation of infertility are to assess the ovarian function.

### 13.1 Ovarian function

Ovarian function can be evaluated by various methods. Regular menstrual cycles are a sign of ovulation in 95% of the cycles. Because the ovaries also "age," however, the regularity of the cycles alone is not enough to characterize ovarian function. The number of follicles in the ovaries decreases from birth. As a result, from the age of 30 onward a slow decline in fertility occurs. This decline parallels the reduction in the number and quality of the follicles and oocytes. The first sign of reduced ovarian activity is the shortening of the follicular phase, which reduces the length of the ovulatory cycle. The decrease in the number of follicles is followed by hormonal changes. Inhibin B is produced by the small antral follicles, and as their number declines, the ovarian output of inhibin B decreases. This is paralleled by a rise in FSH level.

For the everyday practice, there are several tests to assess ovarian reserve. Measurement of the early follicular phase FSH and estradiol levels to determine the FSH/estradiol ratio; measurement of inhibin B or anti-Müllerian hormone levels; or the early follicular phase antral follicle count are options (Table 7). Dynamic tests evaluate the ovaries during clomiphene citrate (CC) challenge or during gonadotropin-releasing hormone agonist (GnRHa) or gonadotropin stimulation.

| Test | Normal Value | Abnormal Value |
|---|---|---|
| Cycle Day 3 FSH | < 10-15 mIU/mL | > 10-15 mIU/mL |
| Cycle Day 3 estradiol | < 80 pg/mL | > 80 pg/mL |
| Inhibin B | > 45 pg/mL | < 45 pg/mL |
| Anti-Müllerian hormone | > 2.7 ng/mL associated with improved oocyte quality as reflected in a higher implantation rate and trend toward better clinical pregnancy rate[56] | Low levels |
| Clomiphene citrate challenge test | FSH < 26 mIU/mL on Day 10 | FSH > 26 mIU/mL on Day 10 |
| Gonadotropin stimulation test | Estradiol level elevation and subsequent decrease | No elevation of estradiol level |
| Antral follicle count on ultrasound | > 3-4 | < 3-4 |
| Ovarian volume on ultrasound | > 3 mL | < 3 mL |

*Note: Different infertility centers use different tests. Cut-off values may differ from center to center on the basis of their experience and results.

Table 7. Cut-off Values* for the Most Commonly Used Ovarian Reserve Tests

On the one hand, the results of these tests will help with designing treatment (to choose the appropriate treatment, stimulation protocol, and gonadotropin dose), and on the other hand they will be useful for counseling the couple. It is very important that a couple undergoing any form of assisted reproduction has realistic expectations (22).

In addition to these tests, it is useful to perform an ultrasound midcycle to assess the ovary and uterus and to document ovulation. Midcycle ultrasound will document follicle growth and allow us to look at the endometrial lining (eg, thickness and type). Ovulation can be documented in several ways. The easiest is to measure a midluteal phase progesterone level. Changes in the basal body temperature, urinary LH kits, luteal phase endometrial biopsy, and serial ultrasounds are alternatives for assessing ovulation.

When the cycles are irregular, other hormonal measurements -- such as testosterone, dehydroepiandrosterone sulfate (DHEAS), 17-OH progesterone, cortisol, prolactin -- as well as thyroid function tests and dynamic evaluation of pituitary function may be necessary for the infertility work-up. If the results of any of these tests are considered abnormal, conducting imaging studies (eg, MRI, CT, thyroid scan) may be the appropriate step.

### 13.2 Ovulation induction, controlled ovarian hyperstimulation

Ovulation induction has a role in the management of patients with anovulation/oligo-ovulation or regular cycles. In the case of oligo-ovulation, the goal is to restore mono-ovulatory cycles.

Various drugs can be used to restore ovulation. Selective estrogen receptor modulators (eg, CC, tamoxifen) are usually administered first. CC is the agent for which most experience has accumulated. It is administered from Day 3 or 5 of the cycle for 5 days. The starting dose is 50 mg, but if needed the dose can be increased by 50 mg daily during subsequent stimulation. Usually, a daily dose > 150 mg is not recommended, as higher doses compromise endometrial development, and pregnancy rates are very low. Ovulation rates are high (80%)

with CC, but cumulative pregnancy rates are only around 40%. The difference between the high ovulation rates and relatively low pregnancy rates is most likely due to the antiestrogenic effects of CC on the periphery, most prominently at the level of the endometrium. If pregnancy does not occur after a maximum of 6 cycles, other options need to be explored (23).
CC stimulation can be combined with ovulation induction with human chorionic gonadotropin (hCG), especially when a spontaneous LH surge cannot be documented. The multifetal gestation rate is about 10% with CC use. CC has relatively few side effects, with gastrointestinal symptoms, visual changes, and hot flashes being more common.
Aromatase inhibitors (eg, letrozole, anastrozole) have been explored recently. Aromatase is an enzyme that regulates the androgen-estrogen conversion. Aromatase inhibitors work by reducing estradiol level and therefore increasing pituitary gonadotropin output (resulting in decreased estradiol negative feedback). Their use is seldom associated with multifollicular development. Pregnancy rates are about 15% to 20 % per cycle. No adverse perinatal outcome following aromatase inhibitor use has yet been reported in the published, peer-reviewed literature, although the authors of a study presented during the American Society for Reproductive Medicine meeting in 2005 reported a higher rate of congenital anomalies with 5 mg anastrozole (24, 25, 26). Notably, letrozole has warned clinicians against prescribing drugs for ovulation induction on the basis of reports of birth defects and spontaneous miscarriages in its safety database (27). Letrozole is not approved for ovulation induction.
Insulin-sensitizing agents have been successfully used to treat infertile patients with PCOS. Metformin (1500 to 2000 mg daily) has been used most widely. With metformin, ovulation can be documented in about 50% to 60% of cases. Metformin can also be combined with CC in CC-resistant cases. Lower miscarriage rates and fewer cases of gestational diabetes have been reported with metformin use. Metformin is a category B drug; no serious adverse effects have been reported with use during pregnancy. Gastrointestinal side effects are often reported upon initiation of treatment. It is a good approach to start with a lower daily dose and slowly increase it to the therapeutic range. Metformin should not be used in women with liver or renal disease. It takes at least 2 to 3 months for insulin sensitizers to take full effect (28, 29, 30, 31, 32).
Gonadotropins can be administered when oral preparations are ineffective or do not lead to pregnancy after repeated attempts. Gonadotropins can be used alone or in combination with oral preparations and are usually started on Day 3 of the cycle at an initial dose of 75 to 150 IU. Cycle monitoring (ultrasound ± estradiol measurement) begins after 5 days of stimulation. When gonadotropins are combined with oral preparations, the pill is initiated first (usually on Day 3) and the injections are administered starting 2 days later. Injections are usually administered on every other day. These protocols can be adjusted depending on the response. Although pregnancy rates are higher following gonadotropin stimulation, the risks for multiple gestations and ovarian hyperstimulation syndrome (OHSS) are increased as well.
Ovulation induction cycles can be completed in different ways. Urinary LH kits can be used to predict ovulation and to time intercourse or insemination. Alternatively, when the lead follicle is around 18 to 20 mm in diameter, human chorionic gonadotropin (hCG) can be administered to induce ovulation. When hCG is used, intercourse or insemination is scheduled 36 to 40 hrs after the injection.

## 14. Intrauterine Insemination

Intrauterine insemination (IUI) further improves the chances of pregnancy. IUI is more effective than intracervical or intravaginal insemination. Its use is especially indicated when

mild male factor or cervical factor infertility is diagnosed. A wide range of pregnancy rates have been reported after insemination (5% to 20% per cycle). Pregnancy rates are higher when gonadotropin stimulation is used in conjunction with IUI. Pregnancy rates are affected by the age of the female partner, semen parameters, tubal status, the presence of endometriosis, and the order of the treatment cycle. The pregnancy and multiple gestation rates are highest with the first treatment cycle. Some even recommend performing the first IUI in a natural unstimulated cycle to avoid multiple gestations and only to proceed with stimulation if the first attempt fails. Usually IUI should not be repeated more than 3 or 4 times. Two exceptions are when donor sperm is used and when the patient has oligo-ovulation; in these cases, a significant number of further pregnancies have been reported in a 5th or 6th cycle. The decision should be made individually, and the availability of IVF obviously influences the decision (33, 34).

## 15. In vitro fertilization, intracytoplasmic sperm injection

The first baby conceived after IVF treatment was born in 1978. Since then, the field has undergone enormous development, and IVF is now routinely used in the management of various forms of infertility. Initially, it was used for the treatment of tubal factor infertility, but today it is used to help patients with male factor infertility, unexplained infertility, genetic problems, and those who fail in vivo treatments.

Early on, IVF was carried out during the patients' natural cycle. Later, CC was added to the protocol to increase efficacy. These cycles were characterized by relatively high cancellation rates as a result of premature ovulation and low pregnancy rates. With the advent of GnRH agonists, antagonists, and different types of gonadotropins, new stimulation protocols have been developed. Cycles with these protocols, by contrast, are characterized by very low cancellation rates, a higher number of oocytes, better-quality embryos, and significantly higher implantation and pregnancy rates.

A typical IVF cycle is made up of 3 parts: stimulation, egg retrieval, and embryo transfer. Stimulation usually consists of pretreatment and stimulation. Pretreatment with oral contraceptive pills or a GnRH agonist allows flexible cycle scheduling and a more simultaneous follicle growth. In the various stimulation protocols, GnRH agonist or antagonist can be given to prevent premature LH surges. The GnRH agonist can be initiated in the luteal phase of the preceding cycle (long protocol) or with the onset of menstruation together with gonadotropins (short, ultrashort protocols). The GnRH agonist initially depletes the pituitary gonadotropin stores ("flare up" effect) before it prevents further FSH and LH release (usually after 7 to 12 days). This initial flare effect is used with the short protocols.

A GnRH antagonist has a different mechanism of action. It competes with GnRH for its pituitary receptors. Upon administration it immediately prevents FSH and LH release. In GnRH antagonist cycles, the antagonist is administered either on Day 6 of stimulation (fixed protocol) or when the lead follicle reaches 14 mm in diameter (flexible protocol).

There are 5 or 6 different stimulation protocols in use by IVF centers. Subtle differences in the management of the pretreatment phase or in the type and dose of gonadotropins do exist between IVF clinics. Several patient characteristics are considered before one decides about the protocol to be used. Typically, age, results of the ovarian reserve testing, and response to previous stimulation help with the decision about the appropriate stimulation protocol (35).

Cycle monitoring (ultrasound and estradiol measurements) usually starts after 5 days of stimulation. When at least 2 follicles reach 17 to 18 mm in diameter, the final steps of oocyte maturation are induced by 5000 to 10,000 IU hCG. In those cycles during which GnRH

agonist downregulation is not applied, the final maturation of the oocytes can be induced with GnRH agonist as well. This method is associated with a lower incidence of OHSS. Oocyte retrieval is scheduled 35 to 36 hours after the final injection.

Oocyte retrieval is an ultrasound-guided vaginal procedure that is performed under intravenous sedation. Oocytes are collected in culture medium and are processed for fertilization. Human tubular fluid was used as an example to design culture medium. Currently, several companies produce culture medium. Use of sequential media tries to satisfy the changing needs of the developing embryo.

Fertilization may occur spontaneously when the sperm number, motility and morphology are within the normal range or can be done using intracytoplasmic sperm injection (ICSI). ICSI is used when the sperm parameters are suboptimal or when fertilization was poor in a previous cycle. During ICSI, the immobilized sperm is transferred through the zona pellucida with a fine glass needle to allow fertilization to take place (36).

The day after the retrieval, the oocytes are checked for signs of fertilization (presence of 2 pronuclei) and are cultured for an additional 2 to 4 days. Transfer usually takes place on Day 3 or 5 after the retrieval. Embryos are assessed on the basis of blastomere number and morphology. Usually 2 or 3 good-quality embryos are transferred. The decision is influenced by the order of the cycle, the patient's age, the number and quality of the embryos, the couple's wishes, and by regulations in those countries where the number of embryos to be transferred is limited. Surplus good-quality embryos can be frozen and stored for later use. To reduce the number of multiple gestations, there is tendency toward transferring fewer embryos. In some countries, the transfer of only a single embryo is allowed. Although pregnancy rates per transfer are lower, following the transfer of 1 fresh and 1 cryopreserved embryo, the cumulative pregnancy rates are comparable to rates following the transfer of 2 embryos. Multiple pregnancies occur significantly less often. An efficient cryopreservation program needs to be in place, however, before one can comfortably offer elective single embryo transfer (37).

This patient has oligo-anovulation; therefore, the assessment of her hormonal status is important. Most commonly, irregular ovarian activity has an endocrine etiology including thyroid disease, hyperprolactinemia, androgen excess, PCOS, premature ovarian failure. Transvaginal ultrasound will assess the morphology of the ovaries (ie, whether they are polycystic or not), myometrium, and endometrium. Serial ultrasound will document follicle growth and allows us to look at the changes in the endometrial lining (eg, thickness and type). Once the etiology of the irregular cycles is known, the appropriate treatment can be planned.

Women with PCOS are at increased for impaired glucose tolerance (and diabetes), dylipidemia, and hypertension. Therefore, the baseline evaluation of these metabolic markers should be part of the work-up for this patient.

Weight loss (life-style modification), CC, or insulin sensitizers could be recommended. At least half of women with PCOS are obese. Obesity is associated with insulin resistance that will further compromise ovarian activity. Weight loss and regular exercise are integral parts of their treatment. Weight loss is associated not only with improved ovarian function but also with lower risk for metabolic complications. CC and insulin sensitizers have both been shown to be effective for ovulation induction among women with oligo-ovulation.

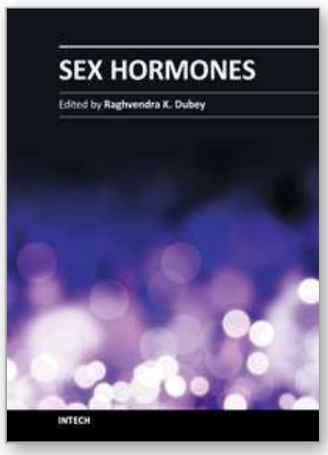
Adding an insulin sensitizer such as metformin would be the next step. A daily dose > 150 mg of CC is not recommended, as higher doses compromise endometrial development, and pregnancy rates are very low. Insulin-sensitizing agents have been successfully used to treat infertile patients with PCOS. Metformin (1500-2000 mg daily) has been used most widely. With metformin, ovulation can be documented in about 50% to 60% of the cases. Metformin can be combined with CC in CC-resistant cases. Lower miscarriage rates and fewer cases of

gestational diabetes have been reported with metformin use. Metformin is a category B drug; no adverse effects have been reported with use during pregnancy. Once follicle growth is achieved, adding hCG can help the timing (intercourse or insemination). Without a mature follicle, however, hCG alone does not work.
The patient's husband has a low sperm count. Therefore, IUI could improve this couple's chances for conception. IVF/ICSI would be recommended if IUI was not successful after 3 to 6 attempts.

## 16. References

[1] Plant TM, et al. The arcuate nucleus and the control of the gonadotropin and prolactin secretion in the female rhesus monkey. Endocrinology 1978;102:52–62.
[2] Schwanzel-Fukuda M, et al. Origin of luteinizing hormone releasing hormone neurons. Nature 1989;338:161–164.
[3] Blackwell RE. Concomitant release of FSH and LH induced by native and synthetic LRF. Am J Physiol 1973;224:170–175.
[4] Knobil E. Neuroendocrine control of the menstrual cycle. Recent Prog Horm Res 1980;36:53–88.
[5] Filicori M, et al. Characterization of the physiological pattern of episodic gonadotropin secretion throughout the human menstrual cycle. J Clin Endocrinol Metab 1986;62: 1136–1144.
[6] Howlett TA, et al. Endogenous opioid peptide and hypothalamo-pituitary function. Annu Rev Physiol 1986;48:527–536.
[7] Reid Rl, et al. Effects of exogenous β-endorphin on pituitary hormone secretion and its disappearance rate in normal human subjects. J Clin Endocrinol Metab 1981;52:1179–1184.
[8] Karten MJ, et al. Gonadotropin-releasing hormone analog design. Structure function studies towards the development of agonists and antagonists: rationale and perspective. Endocr Rev 1986;7:44–66.
[9] Conn PM, Crowley WF Jr. Gonadotropin-releasing hormone and its analogs. Annu Rev Med 1994;45:391–405.
[10] Ron–El R, et al. Gonadotropins and combined gonadotropin– releasing hormone agonist gonadotropins protocols in a randomized prospective study. Fertil Steril 1991;55:574–578.
[11] San Roman GA, et al. A prospective randomized comparison of luteal phase versus concurrent follicular phase initiation of gonadotropin–releasing hormone agonist for in vitro fertilization. Fertil Steril 1992;58:744–749.
[12] Al–Inany H, et al. Gonadotrophin–releasing hormone antagonists for assisted conception. Cochrane Database Syst Rev 2001;CD001750.
[13] Surrey ES, et al. Prolonged GnRH agonist and add-back therapy for symptomatic endometriosis: Long term follow-up. Obstet Gynecol 2002; 99: 709.
[14] Vaitukaitis JL, et al. Gonadotropins and their subunits: basic and clinical studies. Recent Prog Horm Res 1976;32:289–331.
[15] Peters H, Joint A: The Ovary: A Correlation of Structure and Function in Mammals. Berkeley, University of California Press, 1980.
[16] Sasano H, et al. Immunolocalization of aromatase, 17 alpha-hydroxylase and side-chain-cleavage cytochromes P-450 in the human ovary. J Reprod Fertil 85:163, 1989.
[17] Speroff L, et al: Neuroendocrinology. In Clinical Gynecologic Endocrinology and Infertility, 7th ed. Baltimore, Lippincott Williams & Wilkins, 2005.

[18] Fisher B, et al. Tamoxifen for prevention of breast cancer: report of the national surgical adjuvant breast and bowel project P-1 study. J Natl Cancer Inst 1998;90:1371–1388.
[19] Cummings S, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. JAMA 1999;281:2189–2197.
[20] Jayaprakasan K, et al. A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. Fertil Steril 2010; 93: 855-864.
[21] National Center for Health Statistics. Infertility. Available at: http://www.cdc.gov/nchs/fastats/fertile.htm. 2005.
[22] Hillier SG Gonadotropic control of ovarian follicular growth and development Mol Cell Endocrinol. 2001; 179: 39-46.
[23] Hopps CV, et al. The diagnosis and treatment of the azoospermic patient in the age of intracytoplasmic sperm injection. Urol Clin North Am. 2002; 29: 895-911.
[24] Adashi EY. Clomiphene citrate-initiated ovulation. A clinical update. Semin Reprod Endocrinol. 1986; 4: 255-275.
[25] Mitwally MF, et al. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. Fertil Steril. 2001; 75: 305-309.
[26] Healy S, et al. Effects of letrozole on superovulation with gonadotropins in women undergoing intrauterine insemination. Fertil Steril. 2003; 80: 1325-1329.
[27] Biljan MM, et al. The outcome of 150 babies following treatment with letrozole or letrozole and gonadotropins. ASRM/CFAS Annual Meeting; October 15-19, 2005.
[28] Novartis warns doctors on off-label Femara use. Reuters Health. November 30, 2005. Available at: http://www.medscape.com/viewarticle/518136. 2005.
[29] The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovarian syndrome (PCOS). Hum Reprod. 2003; 19: 41-47.
[30] Costello MF, et al. A systematic review of the reproductive system effects of metformin in patients with polycystic ovary syndrome. Fertil Steril. 2003; 79: 1-13.
[31] Glueck CJ, et al Metformin therapy throughout pregnancy reduces the development of gestational diabetes in women with polycystic ovary syndrome. Fertil Steril. 2002; 77: 520-525.
[32] Glueck CJ, et al. Continuing metformin throughout pregnancy in women with polycystic ovary syndrome appears to safely reduce first-trimester abortion: a pilot study. Fertil Steril. 2001; 75: 46-52.
[33] Nestler JE, et al. Strategies for the use of insulin-sensitizing drugs to treat infertility in women with polycystic ovary syndrome. Fertil Steril. 2002; 77: 209-215.
[34] Hughes EG The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: a meta-analysis. Hum Reprod. 1997; 12: 1865-1872.
[35] Dickey R, et al. Effect of diagnosis, age, sperm quality, and number of preovulatory follicles on the outcome of multiple cycles of clomiphene-citrate intrauterine insemination. Fertil Steril. 2002; 78: 1088-1095.
[36] Arslan M, et al. Controlled ovarian hyperstimulation protocols for in vitro fertilization: two decades of experience after the birth of Elizabeth Carr. Fertil Steril. 2005; 84: 555-569.
[37] Palermo GD, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992; 340: 17-18.




**Sex Hormones**

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Sex Hormones not only regulate reproductive function, but they also play a prominent role in the biology and physiology of several organs/tissues and in the pathophysiology of several diseases. During the last two decades, the information on the mechanisms of action of sex hormones, such as estrogens and androgens, has rapidly evolved from the conventional nuclear receptor dependent mechanisms to include additional non-nuclear, non-genomic and receptor-independent mechanisms. This highlights the need to update the current knowledge on sex hormones and their mode of action. Increasing evidence that exogenous/epigenetic factors can influence sex hormone production and action highlights the need to update our knowledge on the mechanisms involved. This book provides a systematic and updated overview of the male/female sex-hormones and their impact in the biology and physiology of various organs. Additionally, the book discusses their positive and negative association with the pathophysiology of various diseases (e.g. osteoporosis, cardiovascular-disease, hypogonadism, reproduction, cancer) and their therapeutic potential.



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