BECKMAN COULTER

Reproductive system in human

Reproductive system in human

Introduction

Human reproduction is a process resulting in the conception of a child, typically involving sexual intercourse between a man and a woman. During intercourse, the interaction between the male and female reproductive systems leads to fertilization of the woman's ovum by the man's sperm. This is followed by a gestation period (pregnancy) and childbirth (labor).

Sexual function and its control is very complex, and proper coordination of all involved physiological processes is necessary for correct reproductive function.

The major organs of the human reproductive system include the external genitalia (penis and vulva) and a number of internal organs such as the gonads (testes and ovaries) that produce both sex cells (sperm and ova) and the hormones necessary for proper development, maintenance, and functioning of involved organs and tissues.

Embryonic development and sexual differentiation

A person’s sex is determined by sex chromosomes at the time of fertilization. In the early stages of embryonic development, the tissues from which the male and female reproductive organs ultimately develop are undifferentiated and consist of two Wolffian ducts, Müllerian ducts, and the undifferentiated gonads.

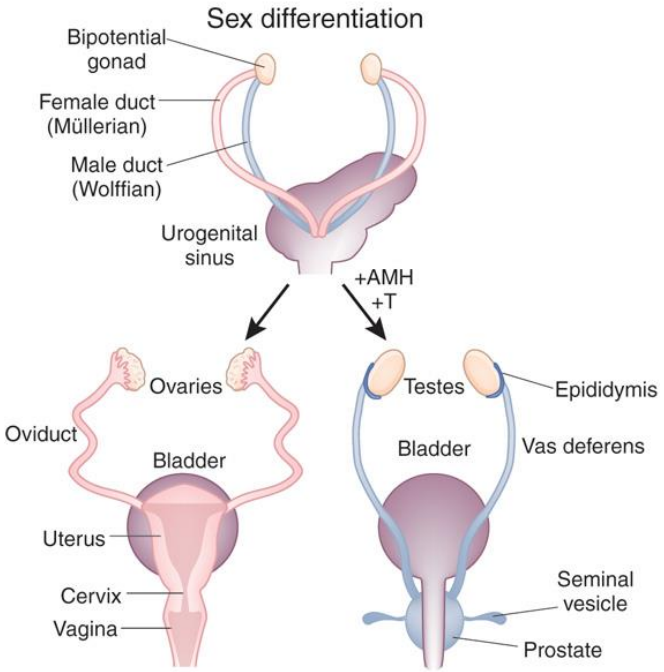
Between the sixth and eighth week of gestation, the gonads develop into testes under the influence of Y chromosome in male fetus, and testicular cells begin to produce anti-Müllerian hormone (AMH) and testosterone.

In female fetus, gonads develop into ovaries in the absence of the influence of AMH and testosterone.

AMH produced by the testes suppresses the Müllerian ducts and prevents development of the uterus and Fallopian tubes in male fetus. Testosterone, together with its derivative dihydrotestosterone (DHT), induces the formation of internal and external male genitalia.

Endocrine hormones are the critical controlling factor in normal sexual differentiation. In the absence of testosterone and DHT, a male embryo with an XY chromosomal pattern will develop female genitalia[1].

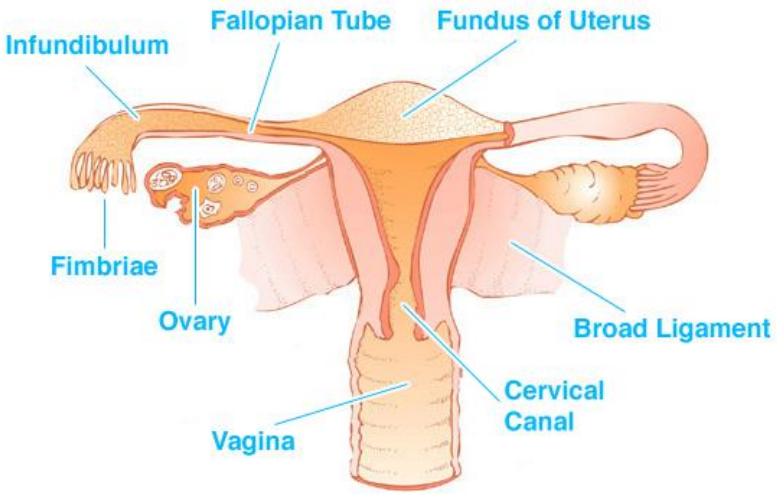
Fig.1: Embryonic development and sexual differentiation



Reproductive function in women

The female reproductive system produces ova (oocyte, egg cells); nourishes, carries, and protects the developing embryo; and nurses the newborn after birth. The system's structures are the ovaries, uterine tubes, uterus, vagina, vulva, and mammary glands.

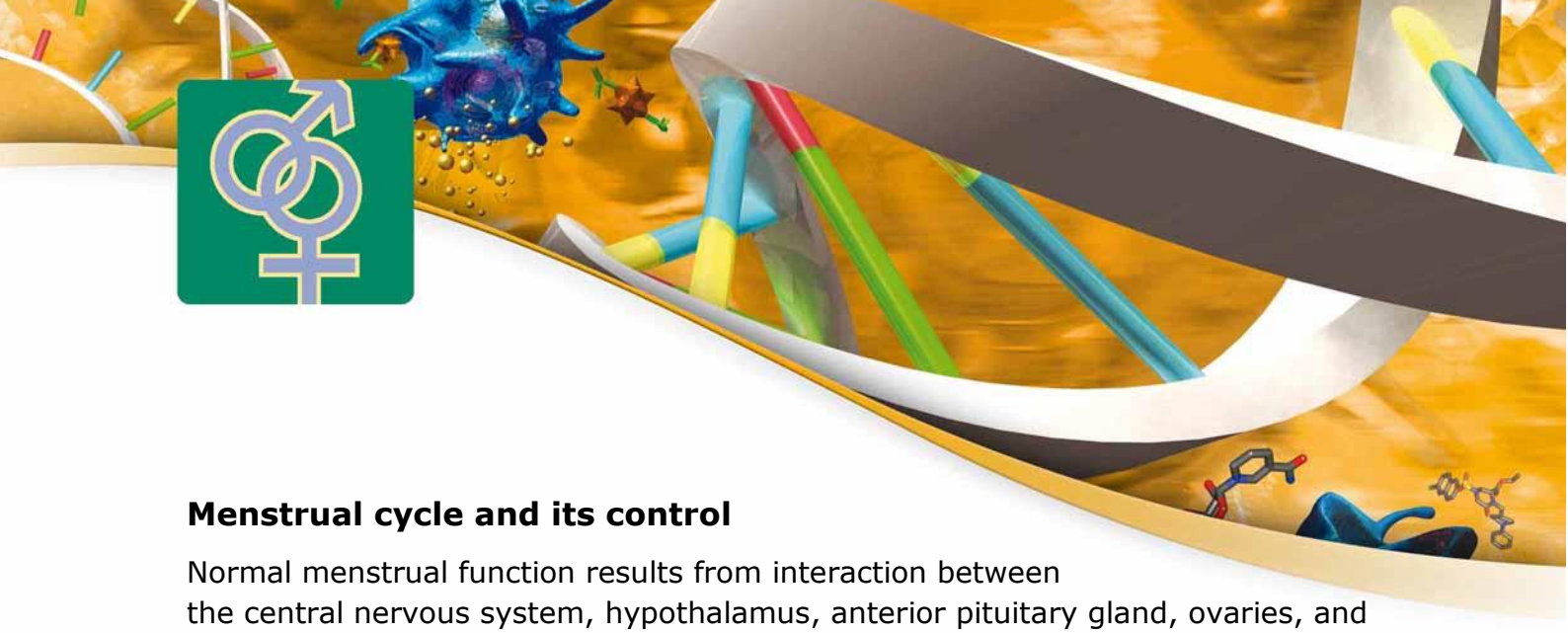
Fig.2: Female reproductive system



The gonads, or ovaries, which are internal in the female (unlike the testes in the male), have the dual function of storing female germ cells, or ova, and producing female sex hormones. Through the regulation and release of sex hormones, the ovaries influence the development of secondary sexual characteristics, regulate menstrual cycles, maintain pregnancy, and induce menopause.

Between menarche (i.e., first menstrual bleeding) and menopause (i.e., last menstrual bleeding), the female reproductive system undergoes a cyclic pattern of change called the menstrual cycle. This includes development of a follicle in the ovary, release of matured ovum during ovulation, and periodic vaginal bleeding resulting from the shedding of the endometrial lining in the uterus.

Follicle - developing ovum surrounded by a cluster of cells



Menstrual cycle and its control

Normal menstrual function results from interaction between the central nervous system, hypothalamus, anterior pituitary gland, ovaries, and associated target tissues. Although each part of the system is essential to normal function, the ovaries hold primary responsibility in regulating the cyclic changes and the length of the menstrual cycle (typically around 28 days).

This regulation is accomplished by the rhythmic synthesis and release of ovarian hormones (estrogens and progesterone) under feedback control from hypothalamic gonadotropin-releasing hormone (GnRH) and the anterior pituitary gonadotropins FSH (follicle-stimulating hormone) and LH (luteinizing hormone).

Hypothalamic-pituitary-gonadal axis

This axis involves the mutual effects of the hypothalamus, pituitary glands and gonads. The hypothalamus secretes GnRH in a pulsatile fashion; GnRH is then transferred to the pituitary gland, where it induces secretion of LH and FSH.

FSH initiates follicular growth, specifically targeting granulosa cells. Together with AMH and inhibin B, it also plays a role in selecting the most advanced follicle for ovulation.

LH triggers ovulation and stimulates development of the corpus luteum.

Ovulation - process by which the matured ovum is released from ovarian follicle

Corpus luteum - structure that develops from the ovarian follicle after its rupture; it produces estrogens and progesterone, i.e. hormones necessary for implantation of fertilized egg in endometrium

In addition to LH and FSH, the anterior pituitary gland secretes a hormone called prolactin. The primary function of prolactin is to stimulate lactation (secretion of milk from the mammary glands) in the postpartum period.

The ovaries produce estrogens, progesterone, and androgens in response to LH and FSH secretion. Estrogens and progesterone down-regulate gonadotropin secretion via negative feedback, either by direct action on the pituitary gland or via inhibition of hypothalamic production of GnRH.

Additionally, estrogens may influence the anterior p
ituitary gland under certain conditions by means of a positive feedback loop. Significantly increased follicular synthesis of estrogens prior to ovulation provokes the anterior pituitary gland to release LH in the so-called "LH surge", starting ovulation.

Estrogens - family of structurally related "female" sex hormones that are necessary for normal female physical maturation, growth of ovarian follicles, maintaining the climate that is favorable to fertilization and implantation of the ovum. They also have a number of extragenital effects, including prevention of bone resorption and regulation of the composition of cholesterol-carrying lipoproteins (HDL and LDL) in the blood.
Three estrogens occur naturally in humans: estrone (E1), estradiol (E2), and estriol (E3). Of these, estradiol is the most biologically potent and the most abundantly secreted product of the ovary. Estriol (E3) is the primary estrogen during pregnancy.

Progesterone – hormone that stimulates development of breast tissue, the cyclic glandular development of the endometrium, and it contribute to the maintenance of pregnancy

Androgens - family of structurally related "male" sex hormones. They may serve as precursors in estrogen synthesis, but they have other functions as well, e.g., stimulation of libido. The most important androgen is testosterone.

Fig 3 shows control mechanism of hormonal synthesis, the typical pattern of hormonal change during the cycle is shown in fig.4.

Fig.3: The regulatory feedback loop of the hypothalamic-pituitary-gonadal axis[19]

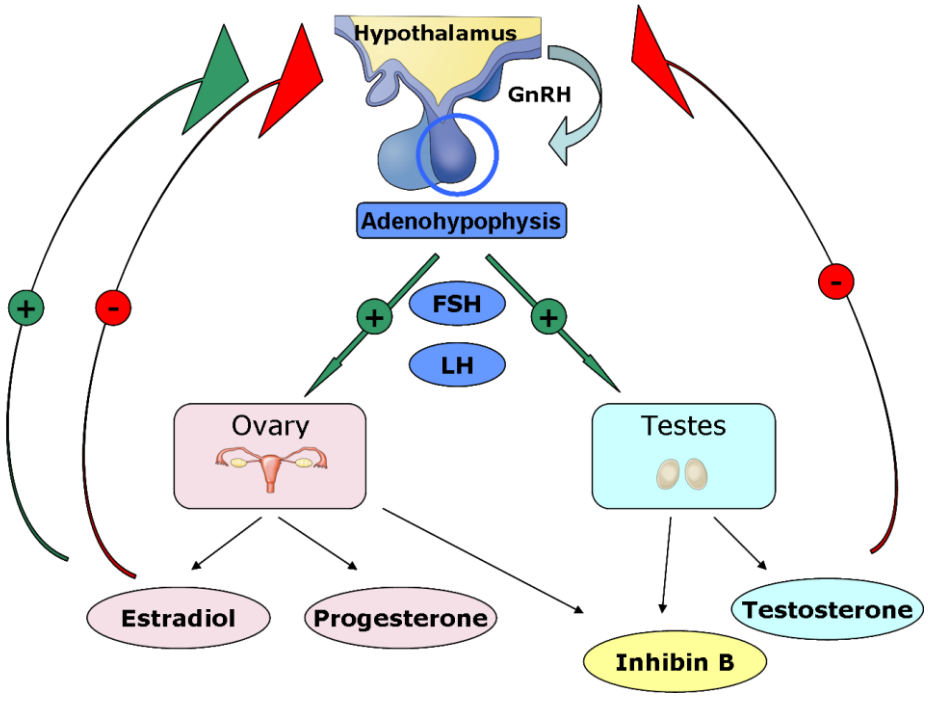
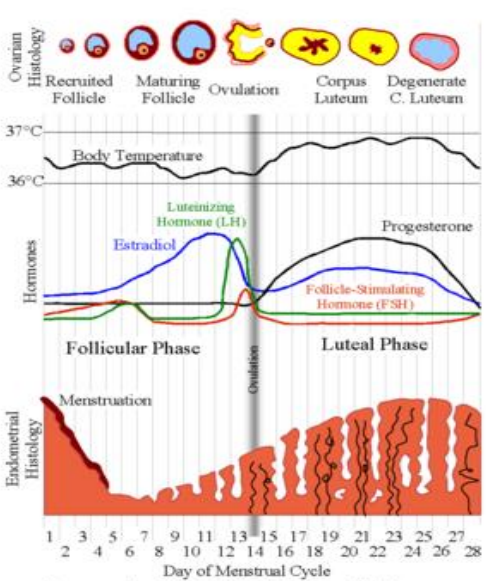


Fig.4: Hormonal, follicular, and endometrial changes during menstrual cycle



Menstrual cycle and ovarian follicle development

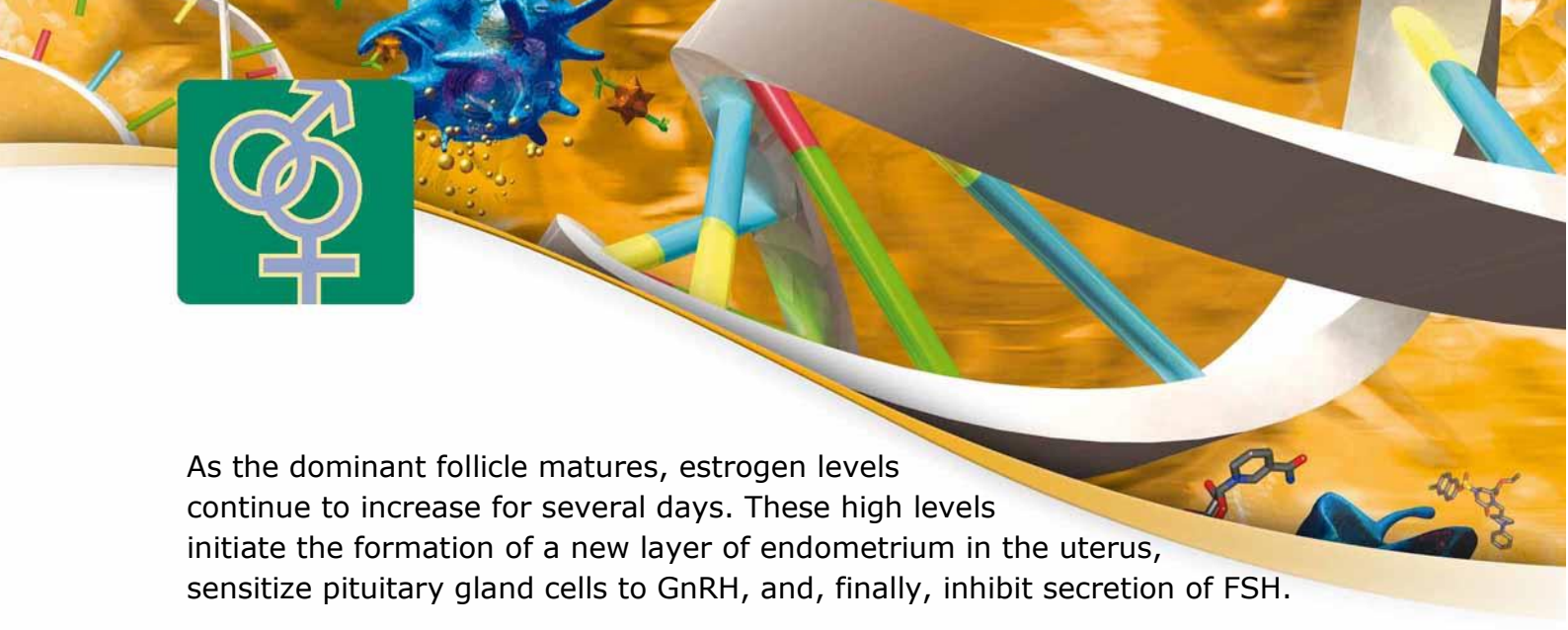
Menstrual cycle can be divided into two phases based on hormonal, ovarian and endometrial changes:

1. Follicular phase (day 1 to 14 of menstrual cycle)
2. Luteal phase (day 14 to 28 of menstrual cycle)

Follicular phase

The follicular phase (or proliferative phase) is the phase of the menstrual cycle during which follicles in the ovary mature. The main hormone controlling this stage is estradiol, under stimulation of FSH.

FSH secretion begins to rise in the last few days of the menstrual cycle, reaching a small peak during the first week of the follicular phase of the subsequent cycle. The rise in FSH levels recruits five to seven antral (Graafian) follicles for entry into the menstrual cycle. These follicles compete for dominance. FSH induces proliferation of granulosa cells in the developing follicles, then stimulates both the secretion of estrogen and the expression of luteinizing hormone (LH) receptors on these cells. Two or three days after LH levels begin to rise (usually by day seven of the cycle), one of the recruited follicles becomes dominant. Estrogen secretion of the dominant follicle increases, suppressing production of GnRH, FSH and LH production and thereby leading to atresia of the non-dominant follicles.



As the dominant follicle matures, estrogen levels continue to increase for several days. These high levels initiate the formation of a new layer of endometrium in the uterus, sensitize pituitary gland cells to GnRH, and, finally, inhibit secretion of FSH.

Ovulation

When estrogen levels reach their peak, positive feedback initiates a significant increase of LH secretion — the so-called "LH surge." The surge induces ovulation, i.e., the process by which ovum is ejected from the follicle, picked up and transported through the fallopian tube toward the uterus. Ovulation normally occurs 30 (± 2) hours after the beginning of the LH surge. The surge also initiates luteinization of thecal and granulosa cells (conversion of the matured follicle to corpus luteum).

The LH surge is accompanied by an increase in FSH secretion.

Luteal phase

The luteal (or secretory) phase is the latter phase of the menstrual cycle. It begins with the formation of the corpus luteum and culminates in either pregnancy or luteolysis. The main hormone associated with this stage is progesterone, whose production is significantly increased during the luteal phase.

After ovulation, the pituitary hormones FSH and LH cause the remaining parts of the dominant follicle to transform into corpus luteum. This continues to grow for some time after ovulation and produces significant amounts of hormones, particularly progesterone and to a lesser extent estrogens. Progesterone, supported by estrogens, plays a vital role in preparing the endometrium for possible implantation of the ovum, and supporting pregnancy in its early phases.

The hormones produced by the corpus luteum suppress production of the FSH and LH, which are in fact necessary for the maintanence of the corpus luteum itself. Consequently, the corpus luteum will atrophy in the absence of conception due to sustained low levels of FSH and LH. The death of the corpus luteum results in the termination of progesterone and estrogen synthesis and a subsequent fall in their values. This drop in ovarian hormone levels causes an elevation in FSH levels, which begins the recruitment of follicles for the next cycle. Subsequent drops in estrogen and progesterone levels trigger the end of the luteal phase, menstruation and the beginning of the next cycle.

The corpus luteum is preserved by implantation of an embryo. Human chorionic gonadotropin (hCG) production maintains functionality of the corpus luteum for eight to twelve weeks, i.e. until placenta takes over these functions.

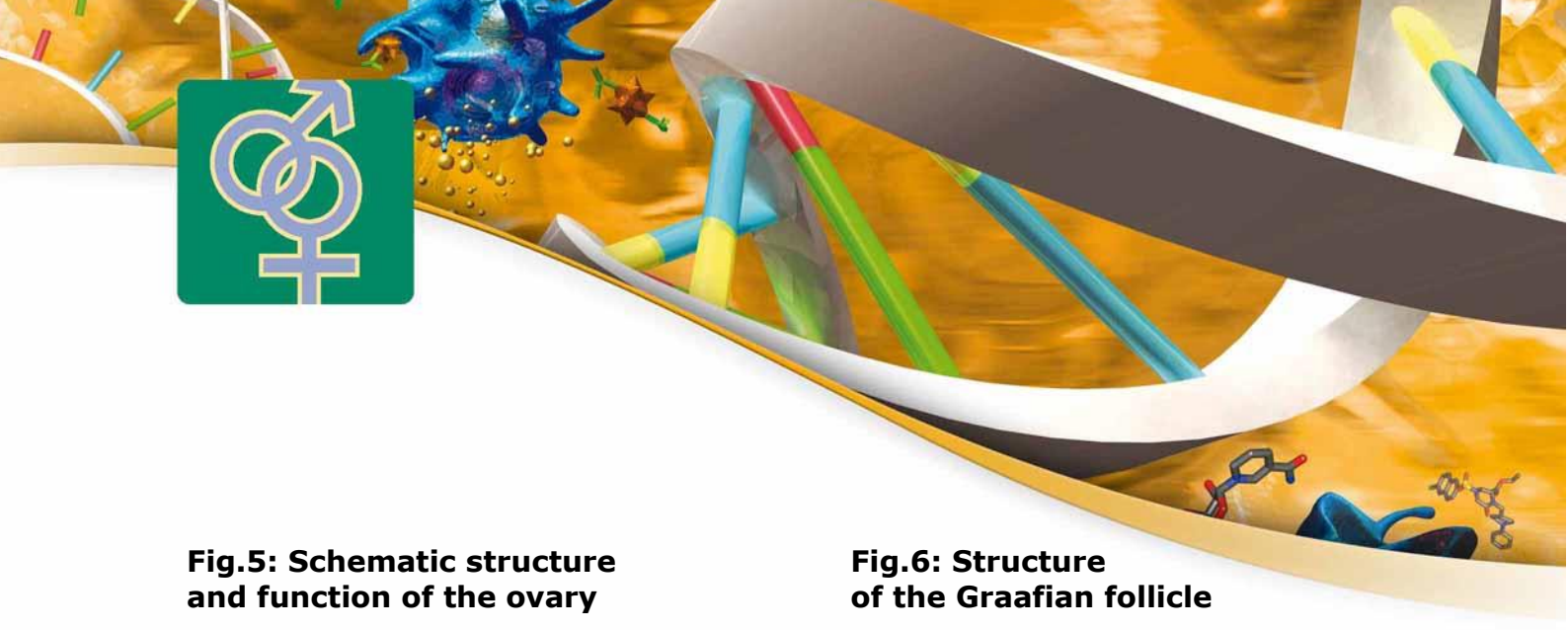


Fig.5: Schematic structure and function of the ovary

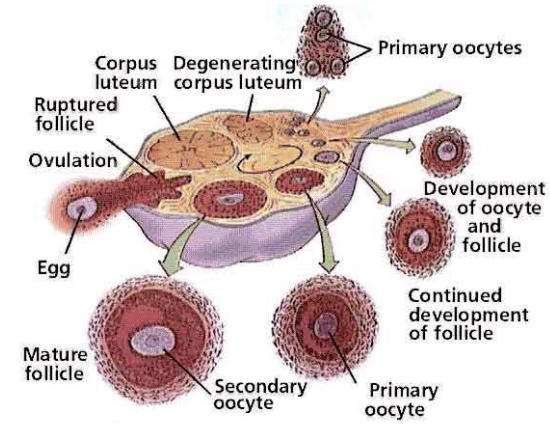
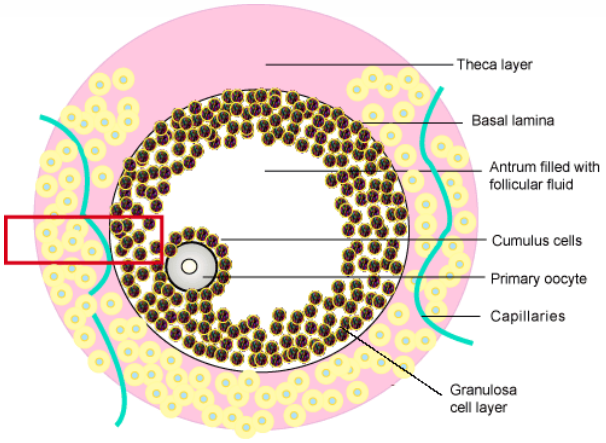


Fig.6: Structure of the Graafian follicle



The tissues of the adult ovary can be divided into four compartments or units: the stroma, or supporting tissue; the interstitial cells; the follicles; and the corpus luteum.

Stroma - connective tissue substance of the ovary throughout which the follicles are distributed.

Interstitial cells - estrogen-secreting cells that resemble Leydig (interstitial) cells of the testes.

Follicle – structure containing the developing ovum, surrounded by a cluster of cells.

Most follicles exist as primary follicles, each of which consists of a round oocyte surrounded by a single layer of granulosa cells and a basement membrane. The primary follicles constitute an pool of inactive follicles from which all the ovulating follicles develop. Under the influence of endocrine stimulation, six to twelve primary follicles develop into secondary follicles. During the development of the secondary follicle, the primary oocyte increases in size, and the granulosa cells proliferate to form a multilayered wall around it. During this time, a membrane called the *zona pellucida* develops and surrounds the oocyte, and small pockets of fluid begin to appear between the granulosa cells. As the follicles mature, FSH stimulates the development of the cell layers. Cells from the surrounding stromal tissue align themselves to form a cellular wall called the *theca.* The cells of the theca become differentiated into two layers: an inner theca interna, which lies adjacent to the follicular cells; and an outer theca externa. As the follicle enlarges, a single large cavity, or *antrum,* is formed, and a portion of the granulosa cells and the oocyte are displaced to one side of the follicle by the fluid that accumulates. The secondary oocyte remains surrounded by a crown of granulosa cells, the corona radiata. As the follicle ripens, ovarian estrogen is produced by the granulosa cells. Selection of a dominant follicle occurs with the conversion of an estrogen microenvironment. The lesser follicles, although continuing to produce some estrogen, atrophy or become atretic. The dominant follicle accumulates a greater mass of granulosa cells, and the theca becomes richly vascular, giving the follicle a hyperemic appearance. High levels of estrogen exert negative feedback on FSH, inhibiting the development of multiple follices and causing an increase in LH production. This represents the follicular stage of the menstrual cycle. As estrogen suppresses FSH, the actions of LH predominate, and the mature follicle (measuring approximately 20 mm) bursts; the oocyte, along with the corona radiata, is ejected from the follicle. The ovum is then picked up and transported through the fallopian tube toward the uterus.

After ovulation, the follicle collapses, and the luteal stage of the menstrual cycle begins. The granulosa cells are invaded by blood vessels and yellow lipochrome-bearing cells from the theca layer. A rapid accumulation of blood and fluid forms a mass called the *corpus luteum.* During the luteal stage, progesterone is secreted from the corpus luteum. If fertilization does not take place, the corpus luteum atrophies and is replaced by white scar tissue called the *corpus albicans;* the hormonal support of the endometrium is withdrawn, and menstruation occurs. In the event of fertilization, human chorionic gonadotropin is produced by the trophoblastic cells in the blastocyst. This hormone prevents luteal regression. The corpus luteum remains functional for 3 months and provides hormonal support for pregnancy until the placenta is fully functional.

Changes in female reproductive function during life

Childhood and puberty

All primordial follicles – 1–2 million - are present in the ovaries at birth. Most of them become athretic and disappear before the onset of puberty, leaving less than 500,000 follicles. Of these, only 400 to 500 undergo the full maturation process resulting in release of matured ovum.

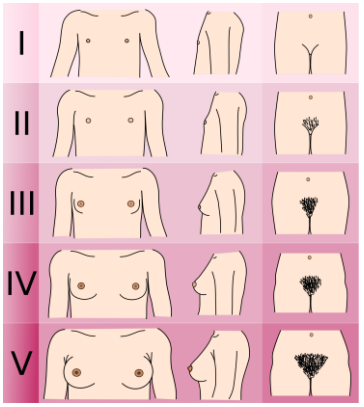
The ovaries function in childhood, when even low production of sex steroid hormones suffices to maintain low levels of LH and FSH. As puberty approaches, there is a significant decrease in the sensitivity of the hypothalamo-pituitary unit to sex steroids, leading to increased secretion of pituitary hormones. Initially, this increased secretion of gonadotropins occurs during sleep, and results increased estradiol levels the following morning. Later in puberty, secretion of LH and FSH is increased to span the entire day (night-time increases still occur, but only during the early follicular phase).

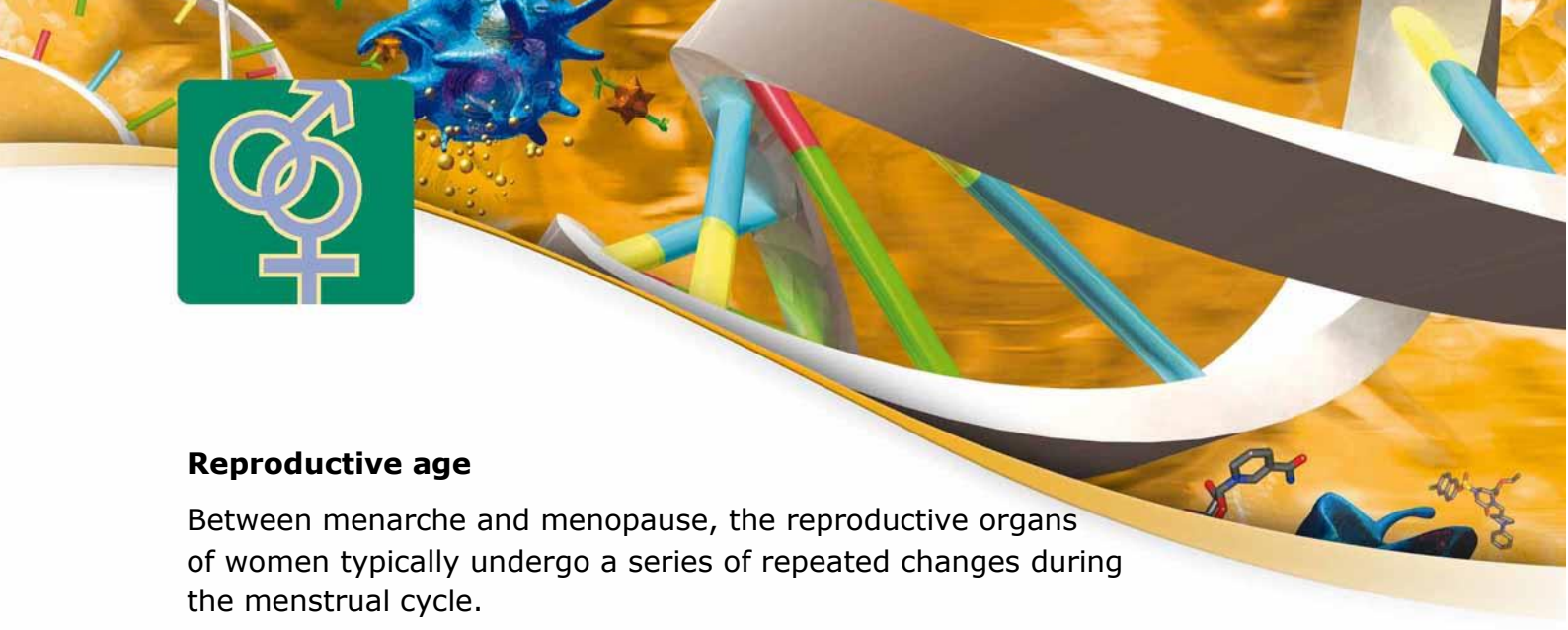
Base levels of estradiol, testosterone and adrenal androgens increase progressively throughout the course of puberty. Increased production of sex hormones induces physical and psychological changes, including development of secondary sex characteristics, accelerated growth and the onset of menarche (first menstrual bleeding). The majority of menstrual cycles remain anovulatory during the first two years after menarche, as the complete balance in hormonal regulation by the hypothalamic-pituitary-ovarian axis is not yet fully established.

Typical changes that appear during puberty are given in Table1. and Fig.7.

Tab.1; Obr.1: Tanner stages of puberty in girls

stage	breasts	Typical age	pubic hair	annual height velocity	other
I	elevation of nipple	<10	villus hair only	5.0- 6.0 cm	adrenarche
II	breast buds palpable, enlarged areola	10-11.5	sparse, slightly pigmented	7.0- 8.0 cm	clitoral enlar-gement; labia pigmentation
III	mammary extends beyond edge of areola	11.5-13	coarser, darker, curled	8.0 cm	acne, underarm hair
IV	nipple mound stacked on areola mound	13-15	adult type, but not beyond pubic area	less than 7.0 cm	first menstruation
V	integral nipple mound	>15	adult type, spreading onto inner thigh	final height reached at age 16	adult genitals





Reproductive age

Between menarche and menopause, the reproductive organs of women typically undergo a series of repeated changes during the menstrual cycle.

This repetitive action is interrupted during pregnancy and in the postpartum period.

Levels of progesterone and estrogens rise continuously throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle. Postpartum suppression of menstrual cycle is connected with breastfeeding and increased levels of prolactin.

In cases of infertility (difficulty or inability to conceive), many different techniques and procedures may be used for treatment depending on the cause of the condition. Hormonal treatment helps in patients with absent or irregular ovulation. If all other methods of assisted reproductive technology (ART) fail, in vitro fertilization IVF) techniques may help.

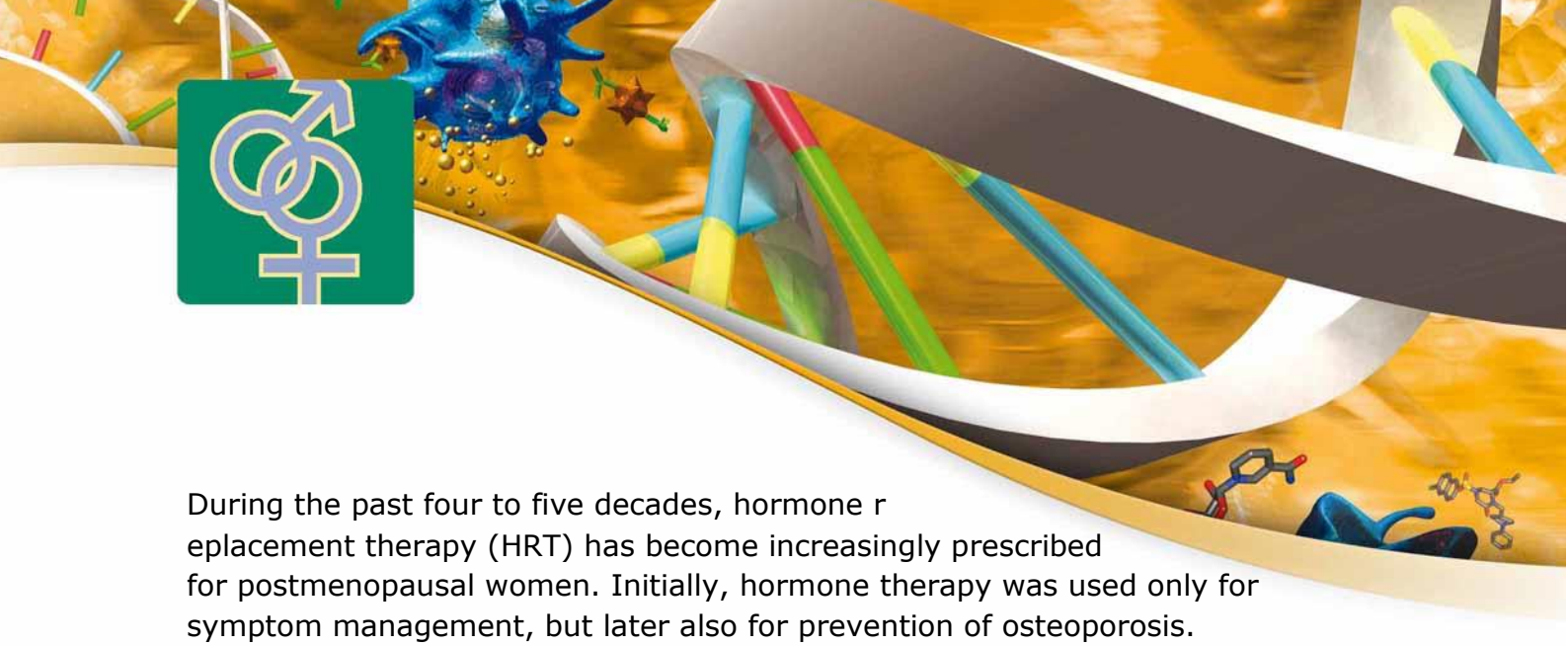
Conversely, steroid contraceptives may be used to prevent conception. Either a combination of estrogens and gestagens or gestagens alone are used to decrease the midcycle gonadotropin surge by inhibiting hypothalamic GnRH release. Follicular development is arrested, circulating levels of LH and FSH are lowered, and other parts of reproductive system are affected as well.

Menopause

Menopause is defined as the day after a woman's final period finishes. This date is fixed retrospectively, once twelve months have passed with no menstrual flow at all. Perimenopause describes the years before and after the final period.

Menopause is a result of the gradual cessation of ovarian function. At that time, ovarian reserve (the capacity of the ovary to provide eggs) ceases. Consequently, ovarian synthesis of estrogens and AMH falls and the FSH levels increase. Estrogens derived from the adrenal cortex, mainly in the form of estrone, continue to circulate in a woman’s body, but they are insufficient to maintain the secondary sexual characteristics in the same manner as ovarian estrogens.

Common symptoms of menopause are “hot flashes”, cold hands and feet, headaches, vertigo, irritability, anxiety, depression, fatigue, weight gain, insomnia, night sweats, etc.



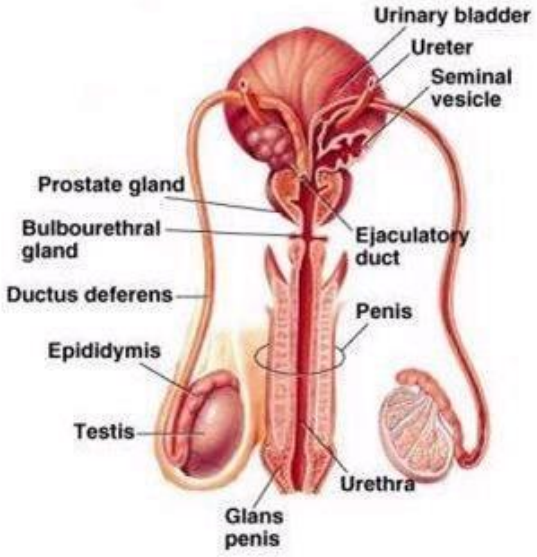
During the past four to five decades, hormone r
eplacement therapy (HRT) has become increasingly prescribed for postmenopausal women. Initially, hormone therapy was used only for symptom management, but later also for prevention of osteoporosis.

Medications based on estrogen or a combination of estrogen and progestin have been used to artificially boost hormone levels. Clinical practice changed dramatically with the publication of two Women's Health Initiative (WHI) studies in 2002 and 2004. These studies found statistically significant increases in the prevalence of of breast cancer, coronary heart disease, strokes and pulmonary emboli in patients on HRT. It seems necessary to find a balance between the risks and benefits of this treatment. Current recommendations are that women take the lowest feasible dose of HRT for the shortest possible time in order to avoid these risks.

Reproductive function in men

The male reproductive system produces semen and sperm, necessary for fertilization of the ovum in the woman's body. The main sex organs, the penis and the testes, are located outside a man's body.

Fig8.: Male reproductive system



The testes have the dual function of producing the male germ cells (sperm) and producing the male sex hormones (androgens).

Androgens - a family of structurally related "male" sex hormones that are necessary for normal male physical maturation, muscle development, bone mass, libido, and sexual performance in men. The testes produce some of them, including androstenedione, dihydrotestosterone (DHT) and mainly testosterone. In target tissues, testosterone may be converted to the most potent androgen - DHT. Several androgens are produced by adrenal gland (less than 5% of total amount), including dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione. These may be metabolized to testosterone and DHT in target tissues.

Male reproductive function is supported by a system of exocrine glands (bulbotheral glands, seminal vesicles and prostate gland) whose secretion form seminal fluid.

Testes also play a vital role in sexual differentiation during embryogenesis, when testosterone stimulates the development and growth of male internal and external genitalia. Fetal testes also produce AMH, which prevents the development of female genitalia.

Starting from puberty, testosterone produced by testes is responsible for the development and maintenance of male secondary sexual characteristics, and sexual functioning (libido and potency).

Spermatogenesis is necessary for fertility.

Male reproductive function and its control

Spermatogenesis

Spermatogenesis is the process by which sperm cells (spermatozoa)developand mature. It takes approximately 70 days. It starts at puberty and usually continues uninterrupted until death, although a slight decrease in quantity occurs with age.

Spermatogenesis takes place within several structures of the male reproductive system. The initial stages occur in the seminiferous tubules of the testes and then progress to the epididymis, where the developing gametes mature and are stored until ejaculation. (fig.9)

Fig.9: Structure of testis and epididymis

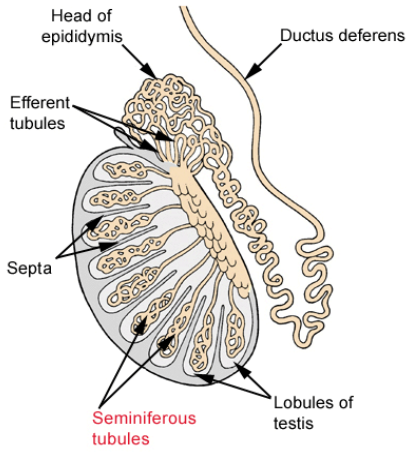
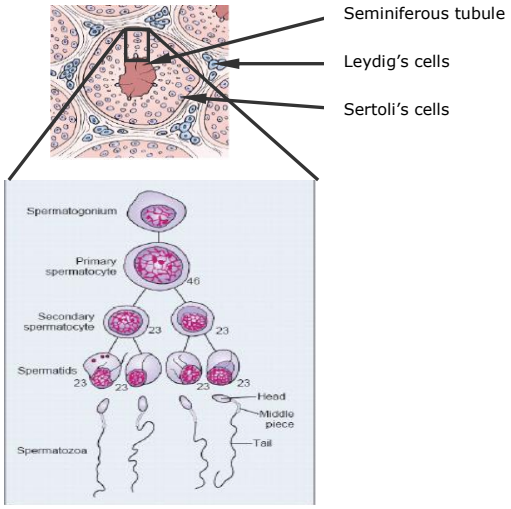


Fig.10: Spermatogenesis



The inner lining of the seminiferous tubules is composed of Sertoli cells, in which are embedded sperm cells in various stages of development (fig.10). Sertoli cells secrete enzymes and other compounds that are required by immature germ cells. They also secrete several hormones including:

AMH – necessary for embryonic development
Estradiol – necessary for successful spermatogenesis
Inhibin B – controls the function of Sertoli cells by inhibiting FSH activity

Both FSH and testosterone are necessary for initiation of spermatogenesis Testosterone in not produced in Sertoli cells, but rather in interstitial Leydig cells (fig.10).

When the sperm grows to full size, it is moved to the epididymis to mature further and to gain mobility.

The sperm count in normal ejaculate is approx. 100-400 million. Infertility occurs when the number of motile sperm is insufficient for fertilization.

Hormonal control of spermatogenesis, hypotalamic-pituitary-gonadal axis

The hypothalamus and the anterior pituitary gland play an essential role in promoting spermatogenic activity. As in women, the synthesis and release of the gonadotropins LH and FSH in the pituitary gland is regulated by gonadotropin releasing hormone (GnRH), which is produced by the hypothalamus and released in pulses (every 2-4 hours) into hypothalamo-hypophysial portal circulation.

LH stimulates the synthesis of testosterone in interstitial Leydig cells. In males, it is therefore also called "interstitial cell-stimulating hormone".

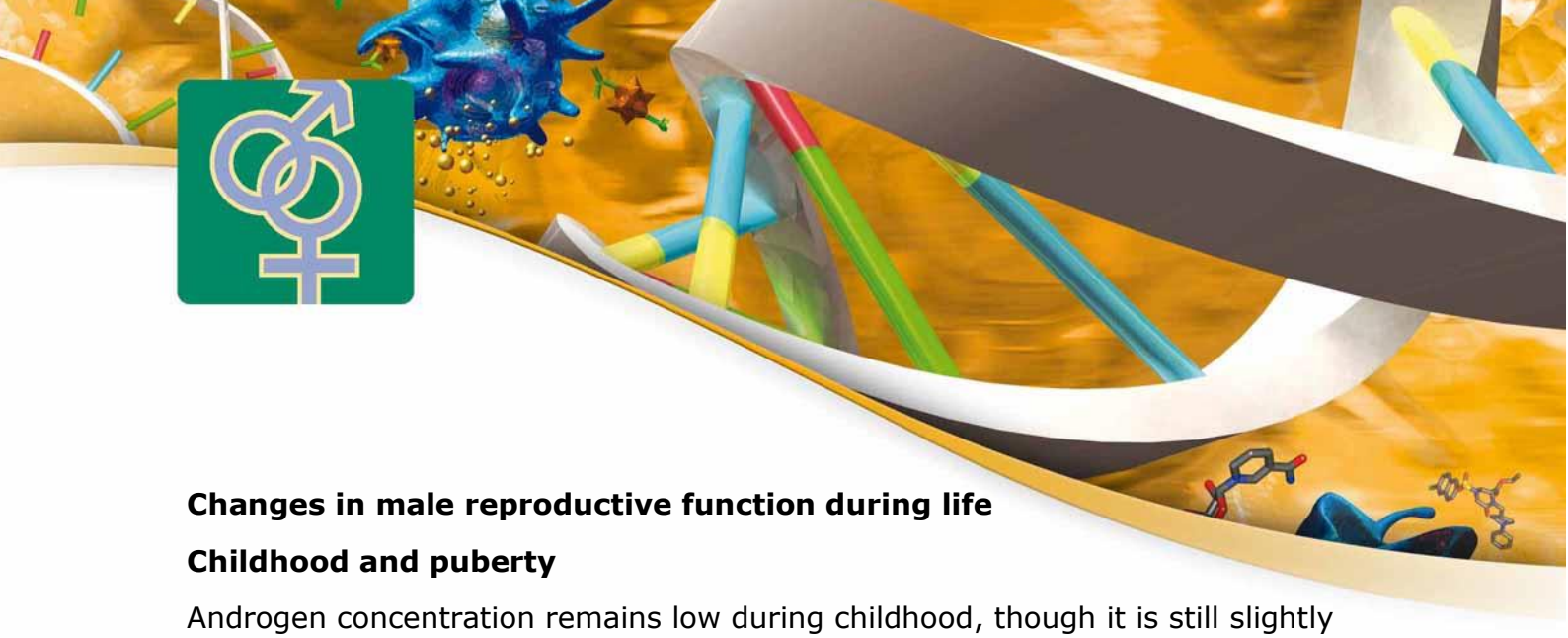
FSH initiates spermatogenesis in Sertoli cells. Under the influence of FSH, Sertoli cells produce androgen-binding protein (the testicular analogue of the blood protein sex hormone binding globuline [SHBG]), plasminogen-activator and inhibin B.

Androgen-binding protein binds testosterone, which is necessary for the full maturation of spermatozoa. Intratesticular testosterone concentration is approximately 100 times higher than in blood. Androgen-binding protein serves to store testosterone and to carry it to Sertoli cells.

Plasminogen activator plays a role in the loosening of spermatozoa from Sertoli cells.

Inhibin B suppresses FSH release from the pituitary gland in a negative feedback loop mechanism.

LH is also regulated by a negative feedback loop. Testosterone directly suppresses its secretion in the pituitary gland and also indirectly by inhibiting the synthesis of GnRH in the hypothalamus.



Changes in male reproductive function during life

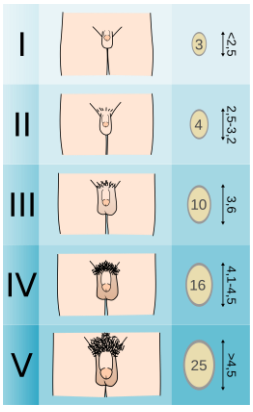
Childhood and puberty

Androgen concentration remains low during childhood, though it is still slightly higher in boys than in girls. Around age 7, concentrations of androstenedione, DHEA and DHEA-S begin to increase. Nevertheless, the onset of puberty comes later, with a decrease in sensitivity of the hypotalamo-pituitary unit to sex steroid hormones. This results in increased secretion of gonadotropins (mainly LH) by the pituitary gland. Initially, this increase occurs during sleep and is associated with production of testosterone by the intersticial Leidig's cells. Hormonal stimulation induces activity of the germ cells and they start to develop into sperm. The testes enlarge in volume with growth of the tubules. Full maturity and spermatogenesis is usually achieved by ages 15-16.

Typical changes that appear during puberty are given in Table 2 and Fig.11.

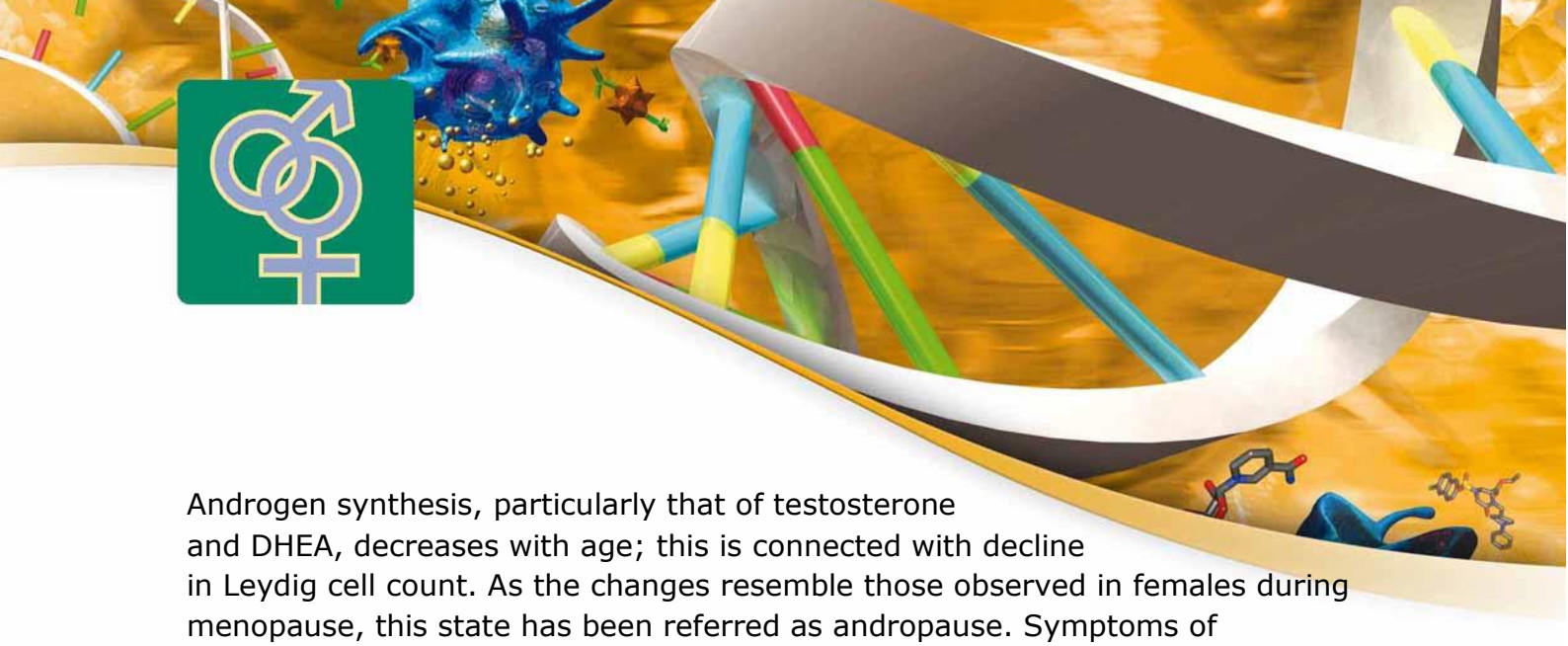
Tab.1; Fig.11: Tanner stages of puberty in boys

stage	Genitals	Typical age (years)	testicle length (cm)	pubic hair	annual height velocity	other
I	infrequent erections	<10	less than 2.5	villus hair only (through age 11)	5 -6 cm	adrenarche
II	scrotum skin thins and reddens; frequent erections	10-11.5	2.5-3.2	sparse, slightly pigmented (ave-rage 12,5)	7.0-8.0 cm	leaner body
III	lengthening of penis	11.5-13	3.3-4.0	coarser, darker, curled (average age 14)	8.0 cm	temporary swell-ing of breasts, voice breaks
IV	thickening of penis, darkening of scrotum skin	13-15	4.1-4.5	adult type, but not beyond pubic area (average age 15)	10.0 cm	acne, underarm hair, voice deepens
V	adult genitals	>15	4.5	adult type, (average 15,5)	full height at 18 or 19	beard, continuing muscle development



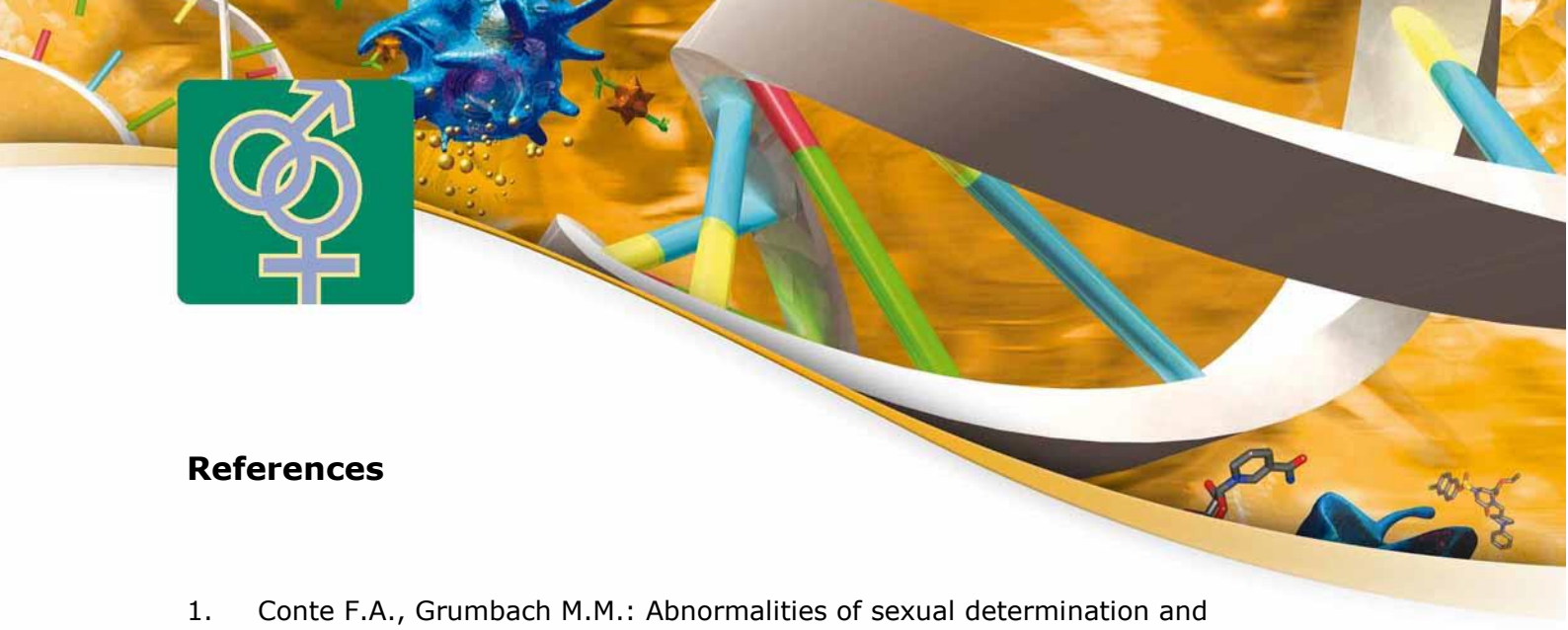
Reproductive age and aging

In principle, men remain fertile from puberty until the end of life. Nevertheless, the male reproductive system becomes less efficient with age, as do other body systems. These changes are more gradual than in women, but there are some similarities.



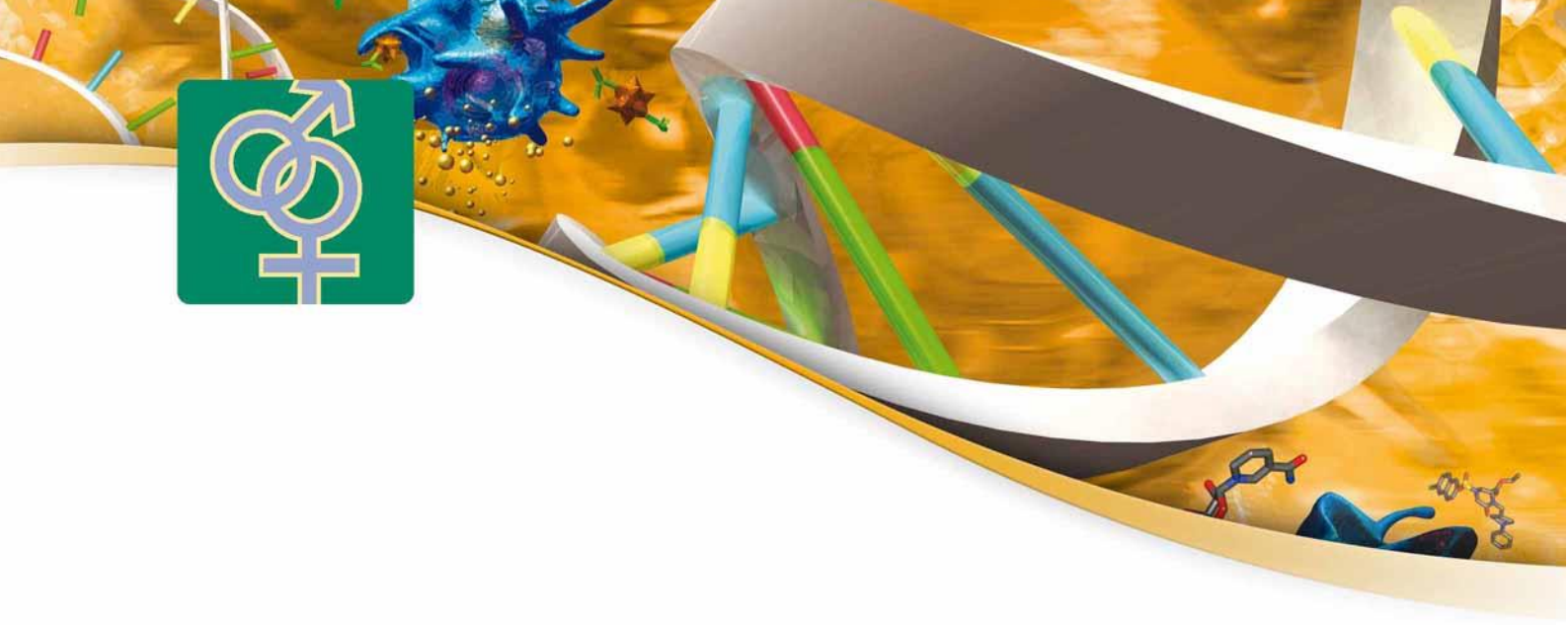
Androgen synthesis, particularly that of testosterone and DHEA, decreases with age; this is connected with decline in Leydig cell count. As the changes resemble those observed in females during menopause, this state has been referred as andropause. Symptoms of andropause include loss of libido and potency, nervousness, depression, impaired memory, inability to concentrate, fatigue, insomnia, hot flushes, and sweating.

Although there is no complete agreement as to whether andropause is a condition that should be diagnosed and treated, questions of increased life expectancy have attracted increasing attention to this issue.



References

1. Conte F.A., Grumbach M.M.: Abnormalities of sexual determination and differentiation. In: Basic and clinical endocrinology, New York:Lange Medical books/McGraw-Hill, 2004, 564-607.
2. Bourguignon, J.P., Linear growth as a function of age at onset of puberty and sex steroid dosage: therapeutic implications, Endocr. Rev.,1988, 9 (4), 467-488
3. Kleerekopper, M., Sullivan, J.M.: Osteoporosis as the model of the long-term clinical consequences of the menopause, Prog. Cardiovasc. Dis., 1995, 38, 181-188
4. Partridge, W.M.: Serum bioavailability of sex steroids, Clinics in Endocrinoloogy and Metabolism, 1986, 15, 259-278
5. Grodstein, F., Stampfer M.: The epidemiology of coronary heart disease and estrogen replacement in postmenopausal women, Prog. Cardiovasc. Dis., 1995, 38, 199-210
6. Carr, B.R.:Textbook of endocrinology, Wilson & Foster Eds., 1992, 733-798
7. Yen, S.S.C.: Chronic anovulation caused by peripheral endocrine disorders, In: Reproductive Endocrinology, Philadelphia, W.B.Sanders Co., 1991, 273-308
8. Lee, J.M., Howell, J.D.: Tall girls: the social shaping of a medical therapy, Arch. Pediatr. Adolesc. Med., 2006, 160(10), 1035-9
9. Hess, R.A, et all: A role for estrogens in the male reproductive system, Nature, 1997, 390, 509-512
10. Massaro, D., Massaro, G.D.: Estrogen regulates pulmonary alveolar formation, loss, and regeneration in mice, Am. J. Physiol. Lung Cell Mol. Physiol., 2004, 287 (6), 1154 -9
11. Warnock, J.K., et all: Combined esterified estrogens and methyltesto-sterone versus esterified estrogens alone in the treatment of loss of sexual interest in surgically menopausal women, Menopause, 2005, 12 (4), 374-84, 2005
12. Hill, R. A., all: Estrogen deficient male mice develop Compulsive Behavior, Biological Psychiatry, 2007, 61, 359
13. O´Malley, B., et all: Steroid hormones: Metabolism and mechanism of action, In: Reproductive endocrinology, Philadelphia, W.B.Saunders Co., 1991, pp. 156-195
14. Adashi, E.Y.,: The ovarian cycle, In: Reproductive Endocrinology, , Philadelphia, W.B. Saunders Co., 1992,.181-237
15. White, P.C.: Vitam. Horm., 49:131,1994



16. Quest Diagnostics/Nichols Institute. The Corning Endocrine Manual, Capistrano, CA: Quest Diagnostics/Nichols Institute, 1996
17. Yen S.S.C.: The human menstrual cycle: Neuroendocrine regulation, In: Reproductive Endocrinology, S.S.C. Yen and R.B. Jaffe, Eds., Philadelphia, W.B.Saunders Co.: 1991, pp. 273-308
18. Losos J.B., Raven, Peter H.; Johnson, George B.; Singer, Susan R. (2002). Biology. New York: McGraw-Hill, pp. 1207-09
19. Burtis C.A., Ashwood E.R.: Tietz Textbook of Clinical Chemistry. 3rd. edition, WB Saunders, Philadelphia, 1999, p. 1615
20. Liu JH, Yen SSC: Induction of midcycle gonadotropin surge by ovarian steroids in women: a critical evaluation, J. Clin. Endocrinol. Metab., 1983, 57, 797-802.
21. Urban RJ, Evans WS, Rogol AD, Kaiser DL, Johnson ML, Velduis JD: Contemporary aspects of discrete peak-detection algorithms. I. the paradigm of the luteinizing hormone pulse signal in men, Endocr. Rev., 1988, 9, 3-37.