Endocrine Evaluation of Reproductive Function in Girls during Infancy, Childhood and Adolescence

Anders Juul\textsuperscript{a} · Casper P. Hagen\textsuperscript{a} · Lise Aksglæde\textsuperscript{a} · Kaspar Sørensen\textsuperscript{a} · Annette Mouritsen\textsuperscript{a} · Hanne Frederiksen\textsuperscript{a} · Katharina M. Main\textsuperscript{a} · Signe Sloth Mogensen\textsuperscript{a} · Anette T. Pedersen\textsuperscript{b}

Departments of \textsuperscript{a}Growth and Reproduction and \textsuperscript{b}Gynaecology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Abstract

Puberty is characterized by a series a hormonal events leading to the attainment of adult reproductive capacity. Clinical manifestations of the pubertal processes include breast development, pubic hair development, menarche and regular menstrual bleedings. Abnormal pubertal development includes a spectrum of disorders such as premature thelarche, premature adrenarche, central and peripheral precocious puberty, adolescent polycystic ovarian syndrome, functional ovarian hyperandrogenism, late-onset congenital adrenal hyperplasia, primary and secondary amenorrhea, and premature ovarian insufficiency. Diagnosis of these reproductive disorders includes biochemical as well as clinical evaluation. The biochemical evaluation of reproductive function includes measurement of basal reproductive hormone levels and dynamic pituitary or adrenal hormone testing. Correct interpretation of such test results requires detailed knowledge on the normal maturational changes in the hypothalamic-pituitary-ovarian and hypothalamic-pituitary-adrenal axes. Changes in basal reproductive hormone levels in infancy, childhood and adolescence as well as the GnRH and ACTH test procedures in girls and adolescents are described in this chapter.

Copyright © 2012 S. Karger AG, Basel

Hypothalamic-Pituitary-Ovarian Axis

Ovarian Development

In the gonad of the XX fetus, the primordial germ cells are transformed at the 10th week of gestation into oogonia which enter first meiotic division. The oogonia stop further differentiation in the diplotene stage, during which they stay until ovulation. Oocytes are formed and peak in number during midgestation (6–7 million) with a subsequent decline [1]. At birth, 1–2 million oocytes are present in the ovaries of the
newborn girl. In postnatal life the number of ovarian germ cells decreases by 1–2% per year by programmed cell death (apoptosis). A minor part of the decreasing number of germ cells is caused by ovulation. From approximately 35–37 years of age, a much steeper rate of germ cell apoptosis occurs concurrent with markedly decreasing reproductive capacity until menopause (fig. 1).

Hormonal Regulation of Ovarian Function
In early pregnancy from around the 4th week of gestation placentally derived human chorionic gonadotropin (hCG) stimulates the undifferentiated gonads. In the presence of the SRY gene, the gonads develop into testes which secrete anti-Müllerian hormone (AMH). AMH causes regression of the Müllerian duct derivatives in the male fetus. In the female gonads which do not produce AMH in early fetal life, the Müllerian structures develop into uterus, oviducts and proximal part of vagina. In the second trimester, the fetal pituitary gland takes over and regulates gonadal function. Hypothalamic gonadotropin-releasing hormone (GnRH) secretion stimulates the pituitary gonadotrophs to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH) through activation of GnRH receptors. FSH and LH act via specific receptors on granulosa and theca cells of the ovary (fig. 2). The serum levels of fetal FSH and LH then decline until birth.

In postnatal life, the hypothalamic-pituitary-ovarian (HPO) axis is activated for the second time at approximately 3 months of age – the so-called minipuberty [3]. During early infancy, basal serum levels of FSH and LH reach those of adult cycling women. After the brief period of HPO activity the HPO axis is silenced during late infancy and childhood resulting in low circulating LH, but clearly detectable FSH levels [4] (fig. 3). With the onset of puberty, the HPO axis is again activated resulting in pulsatile secretion of gonadotropins. The diurnal rhythms of serum LH and
FSH already exist at 5–6 years of age, and serum levels increase before the onset of puberty [5–7]. This is reflected by elevations in urinary FSH and LH concentration before clinical signs of puberty are evident [8]. Altogether, these results suggest that preparation for the onset of female puberty begins already in 5- to 6-year-old girls.

**Inhibin A and B**

The pulsatile FSH and LH secretion stimulates ovarian steroidogenesis under feedback control from circulating estradiol. Granulosa cells also secrete a family of peptides (inhibin-activin-follistatin) in response to FSH stimulation. Two forms of inhibin (inhibin A and inhibin B) exist and consist of two heterodimers linked by disulfide bonds. Inhibin A consists of an α-subunit and a βA-subunit, whereas inhibin B consists of an α-subunit and a βB-subunit. Inhibins suppress pituitary FSH secretion. Serum inhibin B is low or undetectable in Tanner breast stage I. However, measurable levels of inhibin B in more than 60% of prepubertal girls indicate that some gonadotropin-responsive follicular activity is occurring at this early stage. Levels of

---

**Fig. 2.** Preantral ovarian follicle (left part of figure). The oocyte is surrounded by granulosa cells (yellow). The basal membrane (blue) marks the separation between the granulosa cells and the surrounding internal and external thecal cell layers (purple cells). Ovarian steroidogenesis is illustrated in the right part of the figure. Theca cells are stimulated by LH which stimulates steroidogenesis and production of testosterone. Testosterone passes the basal membrane to the granulosa cell where it is aromatized to estrogens, primarily estradiol. Aromatase actively is induced by FSH stimulation of the granulosa cell.
inhibin A increase progressively from Tanner stage I into adulthood. Inhibin A is only detectable in girls after menarche [9, 10], and is therefore a marker of ovulation. During the menstrual cycle, inhibin B levels are highest in the early and late follicular phases, decrease in the periovulatory phase, and are lower in the mid- and end-luteal phases. Serum inhibin A levels are lowest in the early follicular phase, increase significantly in the late follicular phase with maximal levels in the midluteal phase, with a subsequent decrease in the end-luteal phase [10].

**Anti-Müllerian Hormone**

AMH, also termed Müllerian inhibiting substance (MIS), is produced from gestational week 36 and onwards by granulosa cells of the antral follicles. AMH seems to reflect the follicle pool (ovarian aging) in adult women. The biological function of
AMH in the ovary is largely unknown. Serum AMH levels are detectable in girls from 3 months of age with relatively constant levels until 35–40 years of age, followed by a decrease in AMH levels until menopause where AMH becomes undetectable. AMH shows only minor variation across the menstrual cycle [11] depending on the relative ovarian age rather than the chronologic age. Thus, the young ovary secretes higher mean AMH and inhibin B levels to the circulation, and a small rise in AMH during the follicular phase of the menstrual cycle is seen. This is in contrast to the aging ovary which is characterized by low mean AMH and inhibin B serum levels, shorter menstrual cycle lengths, and minimal variation in AMH levels during the cycle, suggesting diminished ovarian reserve. Thus, AMH is considered a promising marker of ovarian reserve [12]. It remains to be seen if pediatric AMH levels predict reproductive function in adult women.

Hypothalamic-Pituitary-Adrenal Axis

Adrenal Development
At approximately the 7th week of gestation, the fetal adrenal cortex is invaded by sympathetic neuronal structures, which differentiate into chromaffin cells which secrete catecholamines. The adrenal cortex consists of 3 zones (zona glomerulosa, zona reticularis and zona fasciculata). Their development depends on adrenocorticotropic hormone (ACTH). The zona fasciculata and reticularis of the adrenal cortex produce cortisol and androgens. The zona glomerulosa of the adrenal medulla secretes primarily aldosterone which is stimulated by the renin-angiotensin system and potassium.

Hormonal Regulation of Adrenal Function
The hypothalamic corticotropin-releasing hormone stimulates pituitary ACTH secretion which is highly pulsatile. ACTH stimulates the adrenal cortex through its specific receptor (MC2R) to secrete glucocorticoids. The steroid biosynthesis pathway is illustrated in figure 4.

The ovary produces all 3 classes of sex steroids; estrogens, progestins, and androgens. The ovary is distinguished from the adrenal cortex by its lack of 21-hydroxylase and 11-hydroxylase reactions. Thus, glucocorticoids and mineralocorticoids are not produced in the normal ovary.

Fig. 4. Steroidogenic pathways in the adrenal and ovary. Cholesterol is taken up by ACTH-dependent mechanisms, and converted into progestins. Progestins are converted into 4 classes of steroids; mineral- and glucocorticoids (exclusively in the adrenal gland), and androgens and estrogens (adrenal and ovary). Cortisol, testosterone and estradiol are end products which are further converted in non-steroidogenic tissues to cortisone (inactive), DHT (potent) and estriol (inactive), respectively. Enzymes are written in blue, and structural changes in the steroids compared to the former steroid metabolite are marked in red.
Steroidogenesis is characterized by a series of enzymatic reactions mediated by steroidogenic enzymes which are members of the cytochrome P450 group of oxidases. All steroids are of similar structure with relatively small chemical differences resulting in major changes in biochemical activity. Conversion of cholesterol to pregnenolone involves hydroxylation at the carbon 20 and 22 positions. The acute regulation of this conversion is controlled by the steroidogenic acute regulatory protein (StAR) which is mediated by ACTH. Following the formation of pregnenolone further conversion can take 2 pathways; the Δ5-pathway via 17-hydroxy-pregnenolone and DHEA, and the Δ4-pathway via progesterone and 17-hydroxy-progesterone.

Steroids are named according to the number of carbon atoms to designate the basic name; pregnane resulting in progestins and corticoids, androstane resulting in androgens, or estrane resulting in estrogens. Glucocorticoids have a C21-structure, and are also referred to as 17-hydroxycorticosteroids. The most important glucocorticoid is cortisol (also known as compound F or hydrocortisone). The interconversion of cortisol and cortisone is catalyzed by 11β-hydroxysteroid dehydrogenase 2, which is present in many peripheral non-steroidogenic tissues including the kidney. Androgens have a C19-structure and are produced in the zona fasciculata and reticularis of the adrenal gland as well as in the gonads. Androgens promote growth and have androgenic effects. Estrogens have a C18-structure, and are converted by aromatase activity from androgens. Estrogen production occurs in the adrenal cortex and gonads, but aromatization of androgens also occurs in peripheral tissues.

DHEAS is the predominant marker of adrenarche in serum which represents an altered pattern of adrenocortical response to ACTH. This is characterized by disproportionately increasing Δ5-steroid intermediates (17OH-pregnenolone and DHEA) compared to Δ4-steroid metabolites (17OH-progesterone and androstenedione). This results from decreasing expression of 3β-HSD in the zona reticularis from 6 to 8 years of age throughout life, which promotes DHEA and DHEAS production. CYP21 expression does not seem to change in the zona reticularis with increasing age, suggesting that adrenarche does not rely on alterations in CYP21 expression. Altogether, the initiation and regulation of adrenarche is poorly understood.

Reproductive Disorders in Girls and Adolescents which Require Biochemical Evaluation

Precocious puberty is defined as clinical signs of puberty before the age of 8 years in girls.

Central Precocious Puberty
Central precocious puberty is caused by activation of the hypothalamic-pituitary-ovarian (HPO) axis, termed central or gonadotropin-dependent precocious puberty (CPP). Central precocious puberty is idiopathic (ICPP) in the majority of cases, but
may be secondary to a brain abnormality like hamartomas, gliomas and hydrocephalus (organic CPP) in 15–20% of cases.

**Peripheral Precocious Puberty**
Alternatively, precocious puberty is caused by an abnormal steroid production in peripheral steroidogenic organs which is not the result of sustained activation of the HPO-axis. This condition is termed peripheral or gonadotropin-independent precocious puberty.

Isolated forms of abnormal precocious puberty like premature thelarche or premature adrenarche are also not caused by central activation of the HPO axis.

**Isolated premature thelarche** is considered a benign, self-limiting condition, characterized by premature breast development, and no other clinical signs of sexual maturation. There are two types of premature thelarche; one type (classical) manifesting itself in the first year of life, following which it tends to resolve by 2 years of age. Another type of premature thelarche manifests itself after 2 years of age and tends to be more persistent. Commonly, breast development fluctuates over a period of time, and can disappear and reappear within months. Premature thelarche is characterized by overnight gonadotropin secretion, but circulating estradiol concentrations are commonly undetectable by conventional immunoassays. A GnRH test will demonstrate a prepubertal LH rise, and a predominant FSH response. The ovaries are small on ultrasound but may contain large follicular cysts which vary in size in synchrony with the fluctuating breast development. Typically, no acceleration of linear growth is seen, and bone age is not advanced. However, this form of premature thelarche may progress into a true central precocious puberty in some cases.

The distinction between central precocious puberty and premature thelarche may not always be straightforward as intermediate slowly progressing forms (thelarche variant forms) exist.

**Isolated premature adrenarche** is characterized by isolated pubic or axillary hair development before 8 years of age in girls, and may be associated with other cutaneous manifestations of androgen excess (like microcomedonal acne, greasy hair and body odor). Also, behavioral changes are seen and bone age may be slightly advanced. Suspicion of adrenal pathology increases, the more bone age is advanced.

**Androgen-producing adrenal tumors** are characterized by a rapidly progressing form of puberty, and serum testosterone are usually high (>5–6 nmol/l). Adrenal tumors are nevertheless extremely rare in children, and may be combined with symptoms of Cushing.

**Congenital adrenal hyperplasia** (CAH) is most commonly due to 21-hydroxylase deficiency (CYP21 mutation), but also other enzyme deficiencies resulting in CAH exist. CAH is typically diagnosed in the neonatal period due to national screening programs, or during the first weeks of life due to clinical symptoms like failure to thrive, salt wasting (hyponatremic crisis) or ambiguous genitalia. However, milder forms of CAH (late-onset CAH) exist, which may present in childhood with varying
degrees of virilization including pubic hair development. Late-onset CAH (CYP21 mutation) is diagnosed by an ACTH test during which 17-OH-progesterone is significantly elevated. Thus, isolated premature adrenarche is an exclusion diagnosis which is relatively common, especially in overweight children and children born small for gestational age. It appears that children born from mothers with PCOS may be at increased risk of premature adrenarche.

**Polycystic ovary syndrome** (PCOS) is a heterogeneous syndrome of unexplained chronic hyperandrogenism and oligo-anovulation, with a polycystic ovary on sonography being one of the alternative diagnostic criteria [13, 14]. Half of the patients lack one or several of the classical criteria (menstrual irregularity, hirsutism, obesity, and polycystic ovaries).

**Functional ovarian hyperandrogenism** (FOH) is usually the source of androgen excess in adolescent girls and is characterized by 17-OH-progesterone hyperresponsiveness to GnRH stimulation or to hCG testing, and subnormal suppressibility of serum testosterone by dexamethasone. However, FOH is often accompanied by functional adrenal hyperresponsiveness, which is characterized by 17-OH-progesterone or DHEA hyperresponsiveness to ACTH [15]. These test are primarily used for research purposes.

**Absent puberty or primary amenorrhea** may result from hypogonadotropic hypogonadism (HH) due to mutations in selected genes (**KAL1, FGFR1, GPR54, PROK2, TAC3, TAC3R**) which account for only 20–30% of cases with HH. Familial delayed puberty may be suspected based on family history once a 45,X karyotype, hyperprolactinemia or anorexia/orthorexia are excluded. Idiopathic HH will result in low FSH and LH levels, and GnRH testing is of limited value in such conditions.

### Evaluation of Basal Serum Hormone Levels

**FSH** and **LH** are glycoproteins which are not single proteins, but consist of a number of heterogeneous forms of varying biological activity. Thus, 20–30 different LH and FSH isoforms are present in the circulation. FSH, LH and hCG are dimers which are composed of 2 glycosylated polypeptide subunits, the α- and β-subunits, that are tightly noncovalently bound. FSH, LH, and hCG share a common α-subunit (92 amino acids), whereas the β-subunits differ in amino acid and carbohydrate content. Sensitive and accurate gonadotropin assays are critical for diagnosis and monitoring of pubertal disorders. Random LH and FSH levels follow a distinct developmental pattern. In prepubertal girls, FSH levels are low, whereas LH levels are usually undetectable by currently available sensitive immunoassays [10]. With the onset of puberty, gonadotropin levels rise gradually in puberty (fig. 3) until the adult cyclic pattern is obtained (fig. 5).

**LH/FSH Ratio**

In prepubertal children FSH levels are higher than those of LH (LH/FSH ratio <1), but half of healthy adolescent children has LH/FSH ratio >1 (fig. 3). This is due to the
fact that the LH/FSH ratio is elevated at ovulation and in the luteal phase of a normal menstrual cycle (fig. 5). Thus, a random LH/FSH >1 in an adolescent girl is not pathological per se, whereas an increased follicular phase LH/FSH ratio is indicative of PCOS in cases of oligomenorrhea/hyperandrogenism. The LH/FSH ratio should not be used as part of the diagnostic criteria for PCOS [13, 14].

**Estrogens**
Estrogens, primarily 17β-estradiol and estrone, are produced in the ovarian granulosa cell following aromatization of their precursors Δ4-Androstenedione and testosterone. Estrogens act through two types of estrogen receptors (ER-α and ER-β). Estrogen production is elevated during minipuberty and with the onset of puberty, whereas levels are typically below the detection limit of commonly used immunoassays in prepubertal children (fig. 6).

Our knowledge of the biological significance of low prepubertal sex steroid levels is hampered by the fact that most available analytical methods are not sensitive enough to assess the low levels of estrogenic and androgenic hormones and metabolites in serum of prepubertal children. Available data on sex steroid levels in prepubertal children differ substantially depending on the assay used and remain controversial. Immunoassays are the most commonly used methods for analysis of estradiol levels. However, for clinical and research applications most immunoassays have insufficient sensitivity resulting in substantial inaccuracy and high intra- as

---

**Fig. 5.** Serum levels of estradiol, LH, FSH and LH/FSH ratio during the menstrual cycles in healthy regularly menstruating young women. Values from individual women are illustrated according to days from ovulation (LH peak). Data derived from Juul et al. [9].
well as interassay variations in the low concentration range. The sensitivity of the most sensitive immunoassays is in the order of 10–18 pmol/l, whereas ultrasensitive recombinant cell bioassays (RCBA) or gas chromatography-tandem mass spectrometry (GC-MS/MS) methodologies have lower detection limits (0.7–1.8 pmol/l) [16–18]. This may be of clinical relevance for instance in girls with premature...
thearche in whom estradiol is usually below the detection limit of commercially available immunoassays, but clearly elevated compared to prepubertal children using a RCBA [16].

**Sex Hormone-Binding Globulin**
Estradiol is bound to sex hormone-binding globulin (SHBG), thus a minor part of circulating estradiol is in its free biologically active form. SHBG is regulated by estrogens, androgens, thyroid hormones, insulin and liver function. SHBG seems to reflect the degree of physiological insulin resistance seen in mid-puberty [19, 20].

**Androgens**
DHEAS levels are low during childhood but start to increase before other hormonal changes of puberty take place, a process termed adrenarche. DHEAS levels are high in patients with adrenal cortical tumors, whereas lesser elevations are seen in patients with congenital adrenal hyperplasia, and moderate elevations are seen in children with precocious adrenarche.

**17-OH-Progesterone**
17-OH-progesterone is produced by the adrenal as well as the ovary. In classical CAH due to 21-hydroxylase deficiency 17-OH-progesterone is clearly elevated, whereas it is only marginally elevated in late-onset CAH.

**Urinary Excretion of Steroids**
The polycyclic carbon ring of steroids is not degraded during metabolism, but is transformed by a series of reductions and hydroxylations. Active steroids are glucuronidated or sulfated and thereby water-soluble, and can be excreted into urine. Generally, glucuronidation reduces the activity of a steroid, but glucuronidated steroids can be deconjugated in peripheral tissues, restoring their biological activity locally. Cortisol is excreted as tetrahydrocortisol (THE) and tetrahydrocortisone (THF) is conjugated with glucuronic acid.

Determination of steroid excretion rates in a 24-hour urine sample is a non-invasive way of estimating the combined output of adrenal and gonadal steroid production. A urinary sample of 5–20 ml is required for urinary steroid profiling by GC-MS/MS methodology. Samples can be sent to the laboratory at room temperature, and should be processed within 3 days. A 24-hour collection allows determination of excretion rates (relevant for diagnosis of Cushing or adrenal insufficiency), whereas a spot urine is sufficient for diagnosis of enzymatic defects in the steroidogenic pathway or tumors.

CAH due to 21-hydroxylase deficiency is characterized by elevated urinary levels of 17-hydroxypregnanolone, pregnanediol, and pregnanetriol, whereas CAH due to 11-hydroxylase deficiency is characterized by elevated urinary tetrahydro-11-deoxycortisol. CAH due to 3β-HSD deficiency show increased urinary levels of
DHEA, 16-hydroxy-DHEA, pregnanetriol and 17-hydroxypregnanetriol. Children with adrenocortical tumors will typically have markedly elevated urinary levels of DHEA. Thus, urinary steroid profiling is useful in a wide variety of clinical conditions. Urinary cortisol metabolites are increased in Cushing’s disease and low or undetectable in adrenal insufficiency.

**Adrenocorticotropic Hormone**

ACTH can be determined in a single blood sample, but is extremely unstable in blood. Thus, care should be taken at blood sampling and the following sample handling procedure should be followed: heparin or EDTA plasma tubes should be placed immediately on ice, centrifuged and the plasma frozen within 15 min. ACTH is elevated in cases of adrenal insufficiency (or pituitary Cushing), but results are typically difficult to interpret.

**GnRH Testing**

Measurement of basal LH and FSH levels is sufficient for some reproductive disorders, but a GnRH test is necessary for diagnosis of CPP. Luteinizing hormone-releasing hormone (LHRH; 100 μg Relefact®) is injected intravenously, and blood samples are drawn before and 30 min after the injection of a bolus. Blood samples are analyzed for LH and FSH. The test is easy to perform, and can be performed in an outpatient setting at any time during the day. The upper normal range of stimulated LH levels in prepubertal children depends on the LH assay, and may vary from 3.3 to 5–6 IU/l. A peak LH level >5 IU/l [21, 22] and/or stimulated LH/FSH ratio >0.66 IU/l [23] has been suggested as cut off limits for a pubertal response during GnRH testing in accordance with the recent consensus guidelines [24] (fig. 7). Furthermore, girls presenting with breast development and a basal LH level above 0.3 IU/l are assumed to have CPP [22].

**ACTH Testing**

A short standard ACTH test using tetracosactrin (the synthetic 1–24 subunit of ACTH) is the most commonly used test. A blood sample is drawn and tetracosactrin (Synacthen®) is injected intravenously as a bolus dose of 250 or 36 μg/kg body weight in infants. Blood samples are drawn 30 and 60 min after injection. Firstly, the blood samples are used for cortisol measurements to rule out adrenal insufficiency. Secondly, steroidogenic metabolites (21-OH-progesterone, 11-OH-progesterone or DHEA) may be analyzed during the short ACTH test to evaluate possible CYP21, CYP11 or 3βHSD defects.

A Synacthen test is easy to perform, and can be performed at any time of the day, does not require fasting or bed rest, and Synacthen can also be administered
intramuscularly. A normal cortisol response during an ACTH test is a rise to >500 nmol/l (or a rise of >250 nmol/l). In neonates, the cortisol responses are higher. Ongoing or recent treatment with glucocorticoids may interfere with cortisol measurements by immunoassays, and chromatographic separation is needed in such cases. Furthermore, use of oral contraceptives may result in erroneously high cortisol levels due to the induction of corticosteroid binding globulin, and should be stopped for 4 weeks before ACTH testing is valid.

Conclusions

In girls with premature breast development initial biochemical evaluation may include:
1. basal FSH, LH, E2, SHBG, inhibin A and inhibin B,
2. GnRH test (FSH, LH),
3. AMH (can be used if granulosa cell tumor is suspected).

In girls with premature pubic hair initial biochemical evaluation may include:
1. basal testosterone, DHEAS, Δ4-androstenedione, 17-OHP,
2. Synacthen test (cortisol, 17-OHP),

Fig. 7. Serum LH and FSH at baseline and 30 min following a GnRH intravenous injection (100 μg LHRH, Relefact). The left panel illustrates a typical prepubertal response to a GnRH agonist challenge with FSH predominance (stimulated LH/FSH ratio <1) and maximal LH level below 5 IU/l. The right panel illustrates a typical pubertal GnRH test with a stimulated LH level above 5 IU/l and a LH/FSH ratio >1.
In girls with *hirsutism* initial biochemical evaluation may include:
1. basal testosterone, DHEAS, Δ4-androstenedione, 17-OHP,
2. Synacthen test (cortisol, 17-OHP),
3. fasting glucose, insulin, HbA1C (or preferably an oral glucose tolerance test).

Urinary steroid profiling may additionally be useful in the above-mentioned conditions.

In girls with *primary amenorrhea* or premature ovarian failure initial biochemical evaluation may include:

**References**


Anders Juul
Department of Growth and Reproduction, Rigshospitalet Section 5064, University of Copenhagen
Blegdamsvej 9
DK-2100 Copenhagen (Denmark)
Tel. +45 3545 5085, Fax +45 3545 6054, E-Mail ajuul@rh.dk