

Physiologic induction of puberty in Turner syndrome with very low-dose estradiol

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Abstract

Many hormone preparations are available for the induction of puberty. We present arguments and evidence that favor the use of physiologic hormone replacement regimens with estradiol and progesterone that mimic the normal changes in production of these natural hormones across puberty. Delaying estrogen therapy until 15 years of age to optimize height potential, as previously recommended, seems unwarranted. This emphasis on stature tends to undervalue the psychosocial importance of age-appropriate pubertal maturation and may be deleterious to bone and other aspects of the child's health. It is now clear that puberty induction can be initiated as early as 12 years of age with very low-dose estradiol (starting doses of one-tenth to one-eighth of the adult dose) without compromising enhancement of growth potential by growth hormone (GH). We give suggestions and recommendations for the induction of puberty in Turner syndrome on the basis of existing data from prospective clinical trials. However, such data are sparse, and there are unanswered questions about optimal treatment schedules for stature, feminization, and uterine and bone development since estradiol percutaneous preparations have only recently become available by prescription in sufficiently low dosages to initiate puberty. © 2006 Elsevier B.V. All rights reserved.

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Although sexual infantilism is one of the most common features of Turner syndrome (TS), 30% or more of girls with TS will undergo some spontaneous pubertal development, and 2–5% may have the potential to achieve spontaneous pregnancy [1–3]. However, over 90% of individuals with TS will ultimately have gonadal failure and require hormone replacement therapy.

The optimal hormone replacement therapy for puberty induction in females with TS is unclear. Common practices in Europe [4] and the US [5] have been explored by use of questionnaires. The majority of responding pediatric endocrinologists in the USA use oral conjugated estrogens to treat hypogonadal girls, with the primary aim of treatment being attainment of maximal adult height. In Europe, most use oral ethinyl estradiol or estradiol, with the primary aim of treatment being satisfactory feminization.

Unexpected cardiovascular risks of oral conjugated estrogen use with and without medroxyprogesterone acetate have recently emerged in postmenopausal women [5]. Whether or not these translate into risks in pubertal and premenopausal females or to other forms of estrogen or progestin replacement is unclear. Indeed, it has been suggested that estrogen protects against the development of atherosclerosis, while having deleterious effects after atherosclerosis has formed. However, this is an area of controversy and active research [6]. While the effects of progestins on breast cancer risk are complex, in contrast to other commonly used progestins, progesterone itself confers no risk for breast cancer and may lessen the small estrogen-related increase in risk in premenopausal women [7,8].

These considerations argue for the use of physiologic hormone replacement that mimics the normal changes in production of the natural hormones estradiol and progesterone across puberty [9]. The use of the natural hormones estradiol and progesterone seem preferable to the many estrogenic and gestogenic hormone preparations that are available. The normal adult estradiol production rate averages 100 µg daily during the follicular phase of the menstrual cycle [10]. Therapeutically, this can be approximated by the administration of oral micronized estradiol in the usual daily replacement dose of 2.0 mg or by intramuscular injection of the usual monthly depot replacement dose of 2.5 mg depot estradiol cypionate (DE2), of which two-thirds is comprised of estradiol and one-third by the fatty acid to which it is esterified. Oral estradiol undergoes about 90–95% first-pass hepatic metabolism and, like other oral estrogens, exerts more extensive effects on hepatic function, such as output of hormone binding proteins and high density lipoprotein, than do systemic doses of estradiol that yield similar estradiol blood levels [11,12].

Progesterone is normally produced after ovulation by the normal corpus luteum at an average rate of 15 mg or more daily over most of its lifespan [13]. Therapeutically, this can be approximated by the oral administration of 200 mg micronized progesterone daily for 10 days. Oral progesterone itself has no known active metabolites [14], and it has less interaction with nuclear receptors other than the progesterone receptor than do other progestins, especially medroxyprogesterone acetate [15–17].

When estrogen therapy is required to induce pubertal development, delaying it until 15 years of age, as previously recommended to optimize height potential [18,19], now seems unnecessary unless diagnosis has been delayed and growth is a priority. These recommendations were made in the absence of data on the dosage at which estradiol effects switch from being growth stimulatory to being growth inhibitory. Emphasis on stature tends to undervalue the psychosocial importance of age-appropriate pubertal maturation [20,21] and may

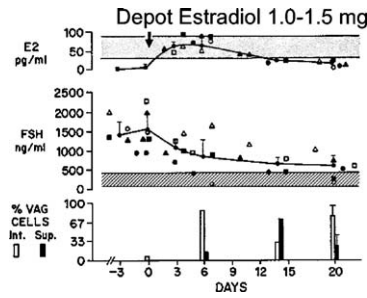


Fig. 1. Responses of girls with Turner syndrome to treatment with depot estradiol (E2). After the administration of depot estradiol (arrow), the plasma estradiol level rises into the adult range and remains there for 2 to 12 days and then returns to baseline at approximately 3 weeks. The biological effects of the dose lag behind the peak blood levels: FSH reaches a nadir and vaginal epithelial cornification peaks between 14 and 21 days. Shaded areas show the normal range of values for mature females in the mid-follicular phase of the menstrual cycle. Percent vaginal epithelial intermediate and superficial cells are shown in the bottom panel. Reproduced from Rosenfield et al., with permission [25].

be deleterious to bone health [5]. Estrogen replacement therapy should be coordinated with the use of GH and individualized for each patient so as to optimize the individual’s goals for growth and pubertal development, taking into account the family history.

It was established some time ago that pubertal hormone replacement with low-dose estradiol can be accomplished without compromising height potential [22–24]. For example, administration of a mid-pubertal dose of DE2 (1.0–1.5 mg) yields mid-follicular phase plasma levels of estradiol for about 10 days and exerts biological effects for at least 3 weeks (Fig. 1) [25]. Monthly administration of these doses in the mid-teenage years permits attainment of predicted adult height (Fig. 2), even though the pace of pubertal development is faster than average, with menses occurring after 3–7 months of treatment. It has also been reported that deterioration of height potential in central precocious puberty generally does not occur in patients whose estradiol levels are less than 20 pg/ml [26].

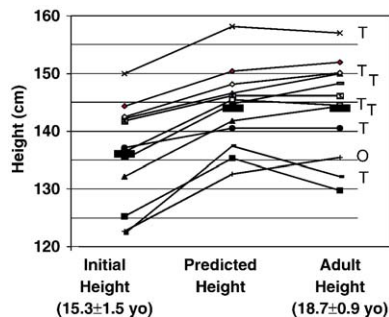


Fig. 2. Height of girls with Turner syndrome in response to long-term treatment with depot estradiol starting at a relatively late age at mid-pubertal doses (1.0–1.5 mg monthly). Adult height was similar to the height predicted at the onset of therapy according to the Bayley-Pinnaeu method. Mean of each group shown by thick bars. Low-dose androgen was given for 0.5–1.0 years concurrently with estrogen: T=depot testosterone 28 mg/m² monthly; O=oxandrolone 0.1 mg/kg daily. Data from Rosenfield et al., [22,23].

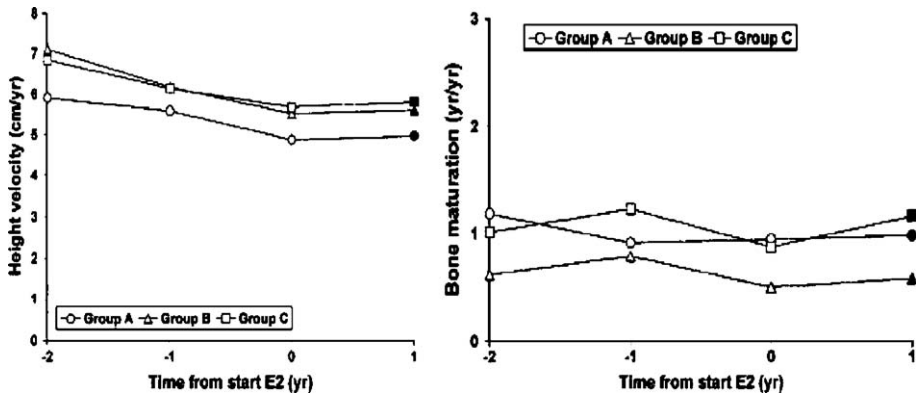


Fig. 3. Height velocity (A) and bone maturation (B) before and after initiation of low-dose estrogen (E2) treatment for girls with Turner syndrome, treated with growth hormone (group A (circle) 0.045 mg/kg/day, group B (triangle) 0.0675 mg/kg/day and group C (square) 0.090 mg/kg/day), who initiated estrogen treatment at age 12 years ($n=47$). The black signs indicate the height velocity and bone maturation after initiation of estrogen treatment. Height velocity in the first year after starting estrogen therapy was not significantly different from that in the previous year. Bone maturation was similarly unchanged after initiation of estrogen therapy. Reproduced from van Pareren et al., with permission [27].

It is now clear that puberty induction can be initiated as early as 12 years of age with very low-dose estradiol (starting doses of one-tenth to one-eighth of the adult dose) without compromising enhancement of growth potential by growth hormone (GH). The greatest experience is with oral administration of micronized estradiol at a starting dose of 5 $\mu\text{g}/\text{kg}$ (which amounts to 250 μg for a 50 kg girl) [27]. Starting this treatment in the 12th year of life does not hinder height velocity in response to GH therapy (Fig. 3); the rate of bone maturation is normal on this dose. The micronized estradiol treatment regimen shown in Table 1 brings about a normal pace of puberty and yields normal adult height in most TS girls.

Recently both injectable depot and transdermal forms of estradiol have been studied at very low dose and may prove to be more physiologic alternatives to oral estradiol treatment [5,9,21,28]. A small randomized controlled study showed that a very low-dose estradiol regimen (Table 2) begun during the 12th year of age stimulated growth and permitted relatively age-appropriate feminization without interfering with the effect of GH on the enhancement of height potential (Fig. 4) [21]. This group gained 5.9 cm more height after starting estrogen than did a matched group of TS subjects (from the National Cooperative

Table 1

Oral micronized estradiol treatment schedule starting at 12 years of age^a

- 1st and 2nd years: 5 $\mu\text{g}/\text{kg}/\text{day}$
- 3rd year: 7.5 $\mu\text{g}/\text{kg}/\text{day}$ + cyclic progestin^b
- Thereafter: 10 $\mu\text{g}/\text{kg}/\text{day}$ till final height
- After achievement of satisfactory (or adult) height, adult dosage begun: estradiol 1–2 mg/day

^a Consider delay of pubertal induction with a later start of GH treatment.

^b Progestin details: dydrogesterone 5 mg/day first 14 days of every month, then 10 mg/day for 14 days when adult height achieved.

Table 2

Very low-dose depot estradiol treatment schedule starting at 12 years of age

- 0–0.5 years: 0.2 mg IM monthly
- 0.6–1.0 years: 0.4 mg IM monthly
- 1.1–1.5 years: 0.6 mg IM monthly
- 1.6–2.0 years: 0.8 mg IM monthly
- 2.1–2.5 years: 1.0 mg IM monthly^a
- 2.6–3.0 years: 1.5 mg IM monthly
- 3.1–3.5 years: 2.0 mg IM monthly
- 3.6–4.0 years: 2.5 mg IM monthly

^a Start micronized progesterone (Prometrium®) 100 mg days 1–7, escalating to 200 mg days 1–10. Start earlier if breakthrough bleeding occurs.

Growth Study database) who had received oral conjugated estrogens at this age ($p < 0.05$). Indeed, the data suggested that this regimen tended to improve adult height in TS patients: those treated in the 12-h year exceeded their 12-year height prediction by 1.6 cm more than those in whom LDE2 treatment was delayed until the 14th year of life (two-tailed $p < 0.07$).

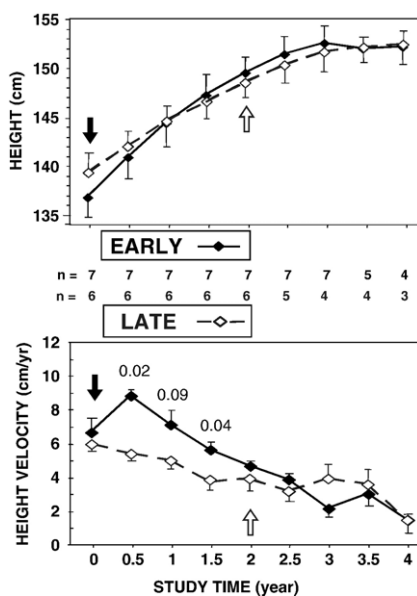


Fig. 4. Height of girls with Turner syndrome in response to long-term treatment with depot estradiol, starting with very low doses (0.2 mg monthly and gradually escalating at 6-month intervals), in combination with GH 0.05 mg/kg/day. GH was begun 0.5 years or more before study year 0. Estradiol was begun (arrows) either early (12th year of age: chronological age 12.5 ± 0.13 years, bone age 11.1 ± 0.42 years) or late (14th year of age: chronological age 14.5 ± 0.1 years, bone age 12.75 ± 0.32 years). Early treatment brought about a significant pubertal growth spurt and tended to bring about a greater adult height. Mean \pm S.E.M. are shown. The number of subjects in each group is shown between panels. Patients achieving sufficient height to discontinue the study did so at different points in the study; 11 were followed to adult or near-adult height. P values shown at peak height velocity. Reproduced from Rosenfield et al., with permission [21].

The most commonly recommended starting dose of transdermal estradiol for the induction of puberty has been 5–10 μg daily [24,29]. This dose range approximates the 133–266 μg monthly amount of estradiol delivered by the 0.2–0.4 mg monthly depot doses of estradiol cypionate that appear to be the most growth-stimulatory. Recently, the use of percutaneous estradiol, typically starting in this dose range, was shown to be associated with 2.1 cm greater height ($p < 0.05$) than the use of oral estrogens, whether oral estradiol, ethinyl estradiol or estrogen–progestin combinations, in a retrospective multivariate analysis of the factors affecting adult height of TS patients in response to GH [28]. An example of a patient treated with transdermal estradiol in this way is shown in Fig. 5.

The plasma estradiol levels achieved on these low doses of transdermal estradiol approximate those seen in normal early puberty [29]. We undertook a pharmacokinetic study that showed that after application of a widely used matrix patch (Vivelle®), plasma estradiol rises about 1 pg/ml (3.67 pmol/l) for every 1.0 μg applied to the skin (Fig. 6) [30].

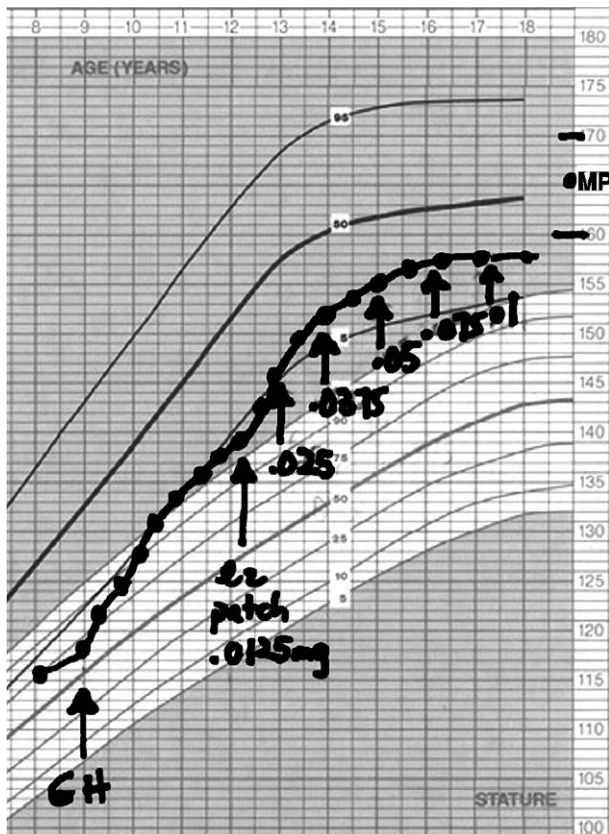


Fig. 5. Growth pattern of a Turner syndrome patient treated with growth hormone and low-dose transdermal estradiol. After 3 years of growth hormone therapy, low-dose estrogen replacement was added at 12 years of age. The starting dose was 12.5 μg daily, and the dose was increased gradually to an adult dose of 0.1 mg daily as shown. This regimen enhanced growth velocity and led to achievement of an adult height within the normal range. Reproduced from Drobac et al., with permission [5].

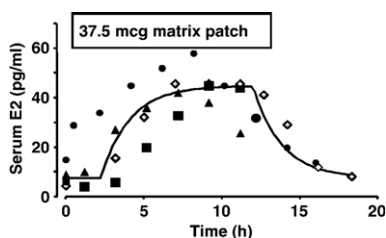


Fig. 6. Estradiol (E2) serum concentrations were determined at baseline and in 2-h pooled samples following application of a matrix patch for 12 h. The pharmacokinetics of transdermal E2 were characterized by a two-compartment open model with zero-order release and first order elimination. E2 was released at a nominal rate from the transdermal patch into a skin depot. Absorption of E2 from the depot compartment into the systemic compartment was described by a first order process. Steady state was achieved approximately 9 h after application of a 37.5 μg patch, and serum E2 concentrations were elevated to a mean of 52.9 ± 17.8 (S.D.) pg/ml.

Thus, application of estradiol doses that average less than 20 μg would be expected to yield plasma estradiol levels below those incriminated in deterioration of height potential [26].

Low-dose estradiol treatment by transdermal application would seem to be a reasonable alternative to very low-dose parenteral estradiol, which is relatively impractical for general use because it requires a compounding pharmacist to dilute the commercially available stock solution to permit reasonably accurate dosimetry. A reasonable very low-dose estradiol transdermal regimen that approximates the plasma estradiol profile shown in Fig. 1, and dose schedule of depot estradiol shown in Fig. 4 would seem to be that illustrated in Table 3.

However, there are unanswered questions about optimal treatment schedules for stature, feminization, and uterine and bone development since estradiol percutaneous preparations have only recently become available by prescription in sufficiently low dosages to initiate puberty. Feminization in response to estradiol is quite variable: breast budding may well not occur on the lowest suggested dose, so may not be acceptable to some girls. On the other hand, menstruation may occur on an estradiol dose below 25 μg daily in some girls, but require 50 μg or more in others.

It is unknown how to best deliver estradiol doses below 14 μg daily. While a European matrix patch can apparently be cut into quarters so the estrogen can be delivered overnight, the US manufacturers recommend against this and our limited experience has yielded

Table 3

A suggested very low-dose transdermal estradiol treatment schedule starting at 12 years of age^a

- 0–0.5 years: 25 μg days 1–7 monthly
- 0.6–1.0 years: 25 μg days 1–14 monthly
- 1.1–1.5 years: 25 μg days 1–21 monthly
- 1.6–2.0 years: 25 μg daily
- 2.1–2.5 years: 37.5 μg daily + progestin^b
- 2.6–3.0 years: 50 μg daily + progestin^b
- 3.1+ years: escalate dosage as necessary every 6 months

^a Consider delay of pubertal induction with a later start of GH treatment.

^b Start micronized progesterone (Prometrium®) 100 mg days 1–7, escalating to 200 mg days 1–10. Start earlier if breakthrough bleeding occurs.

erratic plasma estradiol levels, which suggests that these patches may not be uniformly impregnated with estradiol. It is also unknown what estradiol regimen is necessary for optimal bone mineralization. Our recommendation to administer the lowest transdermal estradiol doses cyclically, rather than continuously, seems to have some physiological precedent since studies in chimpanzees indicate that ovarian estrogen production is cyclic from the onset of puberty [31].

The optimal progestin replacement regimen is also unknown. It may prove best to use a more specific gestogen than medroxyprogesterone acetate. We agreed that it is reasonable to delay the addition of progestin until 2 years after starting estrogen or until breakthrough bleeding occurs. However, it is unclear whether it is necessary to prescribe the 10-day full replacement courses of progestin necessary in adults to prevent endometrial hyperplasia and carcinoma in response to the pharmacologic doses of estrogen that have been common in the past [32]. Indeed, since only half of normal menstrual cycles are ovulatory within 2 years of menarche, it may prove reasonable to prescribe relatively short, low-dosage courses of progestin initially, as presented in Table 3.

While we have given suggestions and recommendations for the induction of puberty in TS on the basis of existing data from prospective clinical trials, such data are sparse. We conclude that detailed studies are still necessary to further optimize treatment for the induction of puberty and to understand how to avoid unwanted side effects and late sequelae of the treatments. In addition, questions of how to proceed once pubertal development is complete also need further study.

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References

- [1] M.I. Boechat, S.J. Westra, B. Lippe, Normal US appearance of ovaries and uterus in four patients with Turner's syndrome and 45,X karyotype, *Pediatr. Radiol.* 26 (1) (1996) 37–39.
- [2] O. Hovatta, Pregnancies in women with Turner's syndrome, *Ann. Med.* 31 (2) (1999) 106–110.
- [3] A.M. Pasquino, et al., Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome, *J. Clin. Endocrinol. Metab.* 82 (6) (1997) 1810–1813.
- [4] W. Kiess, et al., Induction of puberty in the hypogonadal girl—practices and attitudes of pediatric endocrinologists in Europe, *Horm. Res.* 57 (1–2) (2002) 66–71.
- [5] S. Drobac, et al., A workshop on pubertal hormone replacement options in the United States, *J. Pediatr. Endocrinol. Metab.* 19 (1) (2006) 55–64.
- [6] F. Grodstein, J.E. Manson, M.J. Stampfer, Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation, *J. Women's Health (Larchmt)* 15 (1) (2006) 35–44.
- [7] A. Fournier, et al., Breast cancer risk in relation to different types of hormone replacement therapy in the E3N-EPIC cohort, *Int. J. Cancer* 114 (3) (2005) 448–454.
- [8] J.D. Yager, N.E. Davidson, Estrogen carcinogenesis in breast cancer, *N. Engl. J. Med.* 354 (3) (2006) 270–282.
- [9] R.L. Rosenfield, et al., Optimizing estrogen replacement treatment in Turner syndrome, *Pediatr.* 102 (1998) 486–488.
- [10] C. Longcope, J. Pratt, Blood production rates of estrogens in women with differing ratios of urinary estrogen conjugates, *Steroids* 29 (1977) 483–492.

- [11] S. Vehkavaara, et al., Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal women, *Thromb. Haemost.* 85 (4) (2001) 619–625.
- [12] A. Ropponen, et al., Levels of serum C-reactive protein during oral and transdermal estradiol in postmenopausal women with and without a history of intrahepatic cholestasis of pregnancy, *J. Clin. Endocrinol. Metab.* 90 (1) (2005) 142–146.
- [13] T.J. Lin, R.B. Billiar, B. Little, Metabolic clearance of progesterone in the menstrual cycle, *J. Clin. Endocrinol. Metab.* 35 (6) (1972) 879–886.
- [14] C. Munro, et al., Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay, *Clin. Chem.* 37 (1991) 838–844.
- [15] C.P. Thomas, K.Z. Liu, H.S. Vats, Medroxyprogesterone acetate binds the glucocorticoid receptor to stimulate $\{\alpha\}$ -ENaC and sgk1 expression in renal collecting duct epithelia, *Am. J. Physiol., Renal Physiol.* 290 (2) (2006) F306–F312.
- [16] J.A. Kempainen, et al., Distinguishing androgen receptor agonists and antagonists: distinct mechanisms of activation by medroxyprogesterone acetate and dihydrotestosterone, *Mol. Endocrinol.* 13 (3) (1999) 440–454.
- [17] R. Rosenfield, in press. Human skin and progestins. *Hormone and Metabolic Research*.
- [18] S.D. Chernausek, et al., Growth hormone therapy of Turner syndrome: the impact of age of estrogen replacement on final height, *J. Clin. Endocrinol. Metab.* 85 (7) (2000) 2439–2445.
- [19] P. Saenger, et al., Recommendations for the diagnosis and management of Turner syndrome, *J. Clin. Endocrinol. Metab.* 86 (7) (2001) 3061–3069.
- [20] J.C. Carel, et al., Quality of life determinants in young women with turner's syndrome after growth hormone treatment: results of the StaTur population-based cohort study, *J. Clin. Endocrinol. Metab.* 90 (4) (2005) 1992–1997.
- [21] R.L. Rosenfield, et al., Salutary effects of combining early very low-dose systemic estradiol with growth hormone therapy in girls with Turner syndrome, *J. Clin. Endocrinol. Metab.* 90 (12) (2005) 6424–6430.
- [22] R.L. Rosenfield, V.S. Fang, The effects of prolonged physiologic estradiol therapy on the maturation of hypogonadal teenagers, *J. Pediatr.* 85 (1974) 830–837.
- [23] R.L. Rosenfield, Spontaneous puberty and fertility in Turner syndrome, in: R. Rosenfeld, M. Grumbach (Eds.), *Turner Syndrome*, Marcel Dekker, Inc, New York, NY, 1990, pp. 131–148.
- [24] R. Illig, et al., A physiological mode of puberty induction in hypogonadal girls by low dose transdermal 17 beta-oestradiol, *Eur. J. Pediatr.* 150 (2) (1990) 86–91.
- [25] R.L. Rosenfield, et al., The effects of low doses of depot estradiol and testosterone in teenagers with ovarian failure and Turner's syndrome, *J. Clin. Endocrinol. Metab.* 37 (1973) 574–580.
- [26] M. Kreiter, et al., Preserving adult height potential in girls with idiopathic true precocious puberty, *J. Pediatr.* 117 (1990) 364–370.
- [27] Y.K. van Pareren, et al., Final height in girls with turner syndrome after long-term growth hormone treatment in three dosages and low dose estrogens, *J. Clin. Endocrinol. Metab.* 88 (3) (2003) 1119–1125.
- [28] L. Soriano-Guillen, et al., Adult height and pubertal growth in Turner syndrome after treatment with recombinant growth hormone, *J. Clin. Endocrinol. Metab.* 90 (9) (2005) 5197–5204.
- [29] C. Ankarberg-Lindgren, et al., Nocturnal application of transdermal estradiol patches produces levels of estradiol that mimic those seen at the onset of spontaneous puberty in girls, *J. Clin. Endocrinol. Metab.* 86 (7) (2001) 3039–3044.
- [30] L. Zhu, H. Kastrissios, R.R., Pharmacokinetics of transdermal estradiol in teenage girls with Turner's syndrome, *AAPS PharmSci* 4 (2002) W5228 (Abst).
- [31] J. Winter, et al., Pituitary-gonadal relations in infancy: I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee, *J. Clin. Endocrinol. Metab.* 40 (1975) 545.
- [32] J. Woodruff, J. Pickar, Incidence of endometrial hyperplasia in postmenopausal women taking conjugated estrogens (Premarin) with medroxyprogesterone acetate or conjugated estrogens alone, *Am. J. Obstet. Gynecol.* 170 (1994) 1213–1223.