Towards an individualized management of pubertal induction in girls with hypogonadism: insight into the best replacement outcomes from a large multicentre registry

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Abstract

Objective: An evidence-based pubertal induction scheme in hypogonadal girls is still to be established. Interestingly, literature data reports suboptimal uterine longitudinal diameter (ULD) in >50% of treated hypogonadal women, negatively influencing their pregnancy outcomes. This study aims to investigate auxological and uterine outcomes of pubertal induction in girls in the light of underlying diagnosis and therapeutic schemes used.

Design: Retrospective analysis of longitudinal data from a multicentric registry.

Methods: Auxological, biochemical and radiological data were collected at baseline and during follow-up in 95 hypogonadal girls (chronological age>10.9 years, Tanner stage≤2) treated with transdermal 17β-oestradiol patches for at least one year. Induction was started at a median dose of 0.14 mcg/kg/day with a six-monthly increase and was considered completed for 49/95 patients who started progesterone with concomitant oestrogen adult dose.

Results: At the end of induction, the achievement of the complete breast maturation was associated with 17β-oestradiol dose at progesterone introduction. ULD showed a significant correlation with 17β-oestradiol dosage. Final ULD was>65 mm in only 17/45 girls. At multiple regression analysis, pelvic irradiation represented the major determinant of reduced final ULD. After correction for uterine irradiation, ULD was associated with the 17β-oestradiol dose at progesterone introduction. Final ULD was not significantly different from the one assessed after progesterone introduction.

Conclusions: Our results provide evidence that progestins, hampering further changes in uterine volume and breast development, should be introduced only in the presence of a concomitant adequate 17β-oestradiol dose and an appropriate clinical response.

Significance statement

Specific evidence-based Consensus Guidelines for pubertal induction in girls with hypogonadism are still lacking. Moreover, more than 50% treated hypogonadal women show suboptimal uterine dimensions that can negatively influence their pregnancy outcomes. This represents the first longitudinal study on induction of puberty using transdermal 17β-oestradiol in a large cohort of hypogonadal females, counting more than a half patients cancer survivor. According to our results, progestins, hampering further changes in uterine and breast development, should be introduced only in the presence of an appropriate 17β-oestradiol dose and clinical response. If signs of endometrial hyperplasia occur in the presence of underdeveloped breasts or uterus, then a slight reduction in 17β-oestradiol should be taken into consideration to delay progesterone start.

Introduction

Pubertal induction in girls with hypogonadism aims at mimicking natural puberty, in terms of physical and psychological development. Induction of puberty in hypogonadal girls needs to be carefully performed to assure an adequate growth spurt, an adult height consistent with mid-parental height (MPH), an appropriate bone mass accrual, the development of secondary sexual characteristics and cognitive functions, the uterine maturation and the occurrence of menstrual bleeding (1; 2; 3). In addition, pubertal induction optimization should aim at a complete uterine development, thus offering eventual future opportunities of conception that were not even considered possible in the past (1; 3). Indeed, one of the most important effects of oestrogen is to promote uterine development from a tubular structure into a pear-shaped mature configuration leading to an increase in size and volume (4), with the most part of uterine development occurring during puberty and sometimes lasting after menarche. In the absence of an appropriate

oestrogen exposure, the uterus remains hypoplastic or even apparently absent at pelvic ultrasound. Moreover, in childhood cancer survivors (CCS) patients previously treated with pelvic radiotherapy, uterine maturation is further hampered by the known detrimental effect of irradiation (5). As aforementioned, reproductive technology allows women with hypogonadotropic hypogonadism (HH) and even some with primary ovarian insufficiency (POI) to achieve parenthood, the latter with fertility preservation or egg donation (6; 7). In this context, poor uterine development (immature uterine shape, poor endometrial thickness and deficient uterine vascularity) represents one of the contributing factors for negative pregnancy outcomes (4; 5; 8; 9).

Up to now, no studies have been conducted in the specific setting of CCS with chemo- and radiotherapy-induced hypogonadism: the clinical approach to this selected class of patients, indeed, is usually generalised from analyses involving patients with gonadal failure of different aetiologies rather than tailored on their specific clinical features and needs (10).

To date, 17β-oestradiol appears to be theoretically the first choice to induce puberty, as it is a precautionary principle to replace any given endocrine deficiency with the bioidentical hormone, whenever possible (3; 11; 12; 13; 14). Moreover, data from females with hypopituitarism receiving combined oestrogen and GH treatment indicate a markedly greater impairment of GH-mediated insulin-like growth factor 1 (IGF-I) synthesis with EE than with 17β-oestradiol (15), with a smaller inhibitory effect observed by transdermal vs oral 17β-oestradiol route (16): an aspect to take into account especially in patients under concomitant recombinant growth hormone (rGH) therapy for short stature. In the aim of being as physiological as possible, 17β-oestradiol replacement therapy gives the advantage of being monitored according to oestradiol serum levels that can be easily measured during treatment (17).

The administration of 17β-oestradiol can be either oral or transdermal (TD) (3). The theoretical benefit of the TD route is the lack of intestinal absorption variability and the avoidance of the first-pass effect in the liver thus resolving the consequent problems of variable bioavailability, production of hepatic clotting factor and accumulation of non-physiologic oestrogens, usually observed after the oral route (18). Moreover, the matrix composition of the new generation TD patches allows the cutting into pieces in order to start induction at very low doses even modulating the duration of attachment thus mimicking the normal diurnal/nocturnal variations in 17β-oestradiol (17; 19).

Although oestrogen-only pubertal induction is possible, an unopposed oestrogen regimen exposes to a higher risk of future uterine cancer and endometriosis occurrence. At some point, progesterone should be added to shift the endometrium to the secretory stage preventing endometrial hyperplasia and assuring menstrual bleeding (20; 21). To date, in all the combined oestrogen/progesterone regimens available in literature, the latter is introduced after a pre-set period (usually 18-24 months of oestrogen replacement therapy - ERT) or at bleeding occurrence, without any individualization regarding breast and uterine ERT response. However, there is some suggestion that an early administration of progesterone, especially the more androgenic agents, may compromise future breast and uterine growth and development (22; 23).

The optimal oestrogen/progesterone induction regimen, capable of being both physiological and effective, remains to be established on an evidence base.

At this aim, we conducted an explorative investigation into auxological and uterine outcomes of pubertal induction of girls with hypogonadism according to the different underlying diagnosis and therapeutic schemes used.

Patients and Methods

In March 2020 a multicentre register was created in order to collect clinical and therapeutic data of girls affected by hypogonadism (either HH and POI) in different hospitals in Italy. At the present, four centres have accepted to participate:

- Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy;
- IRCCS Istituto Auxologico Italiano, Milan, Italy;
- Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy;
- Bambino Gesù Children's Hospital, IRCCS, Rome, Italy.

Patients

In October 2021, the register included the retrospective data of 106 girls with POI or HH of all causes (either genetically inherited or acquired, isolated or associated with other multiple pituitary hormone deficiencies), aged >10.9 years and treated with EP therapy for pubertal induction from January 2000 to October 2021. Parental written informed consent was obtained for all study patients. The present Registry and its use for scientific purposes have been approved by the Ethic Committee of Milan, Area 2, in the session of 07/04/2020 (authorization number 265_2020). All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Out of 106 patients included, in the present analysis we considered only the 95 girls treated with TD 17β-oestradiol with a follow-up of at least one year from ERT start. Patients with shorter follow-up, treated with oral oestradiol, with uterus aplasia or arrested puberty (Breast Tanner stage >2) were excluded.

Methods

For exploratory purpose, we retrospectively analysed longitudinal data retrieved from the registry. For all study patients auxological (height, weight, BMI, growth velocity and Tanner stage), biochemical (serum oestradiol levels) and radiological (hand and wrist X-ray and pelvic ultrasound) data were collected at baseline (ERT start) and during every 6 months' follow-up according to the clinical practice of the specific Centre. Standing height (HT) was measured with a Harpenden stadiometer. Height, weight and BMI were expressed as standard deviation scores (SDS) according to World Health Organization (WHO) Growth Charts (24).

In all centres, pubertal development was assessed by Paediatric Endocrinologists according to Tanner stages (25). The time between induction start and breast Tanner stage III (B3), IV (B4) and V (B5) achievement was assessed and indicated as ΔB3, ΔB4 and ΔB5, respectively.

Due to the observational and multicentric design of this study, different methods of hormone measurement have been applied. Nonetheless, in most of the cases, 17β-oestradiol concentrations were measured by electrochemiluminescence immunoassay "ECLIA", with a lower limit of detection of 5 pg/mL. For the purposes of statistical analysis, 17β-oestradiol values below the reference limit were estimated as 5 pg/ml.

Hand and wrist X-ray scan for bone age was assessed according to the standard of Tanner-Whitehouse III (26) or Greulich & Pyle Atlas (27). Data about uterine longitudinal diameters were obtained from pelvic ultrasounds.

Mid-Parental Height (MPH) was calculated as [(mother height + father height) -12.5]/2 for all study patients (28). Mid-Parental Height distance was defined as the difference between the

recorded height SDS at different points of assessment (at baseline and at adult height, AH) and MPH SDS (HT-MPH and AH-MPH, respectively).

Adult height was considered in the presence of a height velocity < 2 cm/year or with a hand and wrist X-ray showing complete epiphyseal fusion.

Uterine maturity was defined in the presence of longitudinal diameter length over 65 mm at pelvic ultrasound, according to the normative data by Griffin et al (29). Nonetheless, taking into account the relationship between transverse uterine diameter increase and oestrogen response (30), we considered the change from tubular (pre-pubertal) to a "pear-shaped" morphology with the parallel increase of the corpus/cervix ratio above 2:1 as markers of appropriate oestrogen replacement therapy (ERT) response (4).

All patients underwent TD 17β-oestradiol therapy at incremental dosage every six months according to the clinical practice of the specific Centre and, in the absence of specific evidence-based Consensus Guidelines on the subject, following the proposed regimens available in literature (11; 12; 13). Patches were divided and conserved as previously described (31).

Induction was considered completed in all patients who have:

- introduced combined oral contraceptive (COC) therapy;
- introduced progesterone during TD 17β-oestradiol at least at 50 mcg/day or 1 mcg/kg/day with 6 months follow-up.

If available, data about uterine longitudinal diameter after COC introduction were collected.

Statistics

Continuous variables are showed as mean and standard deviation (SD) in case of normal data or mean and interquartile range (IQR) otherwise. Shapiro-Wilks was used to test if the continuous

variables followed a normal distribution. Categorical variables are showed as absolute and relative frequencies. ANOVA test (or nonparametric Kruskal Wallis test) was applied to compare the continuous variables among groups, while Chi-squared (or Fisher's exact test) for categorical ones. Moreover, paired t-test (or Wilcoxon signed rank sum test) was used for comparisons of continuous variables for dependent data. To investigate the correlation between two continuous variables we calculated Pearson's index (or Spearman's index). Moreover, to study the correlation between a continuous variable and a dichotomous variable the point-biserial coefficient was measured. Finally, considering only the subjects with completed induction, we implemented different multivariate linear regression models. The first one to investigate the determinants of AH (SDS), the second one to evaluate the relationship between several therapy characteristics and uterine longitudinal diameter (ULD). In the latter, we performed a multivariate regression model for each variable of interest, including known clinical confounders: CancerPOI (pelvic RT), Tanner stage at baseline, baseline ULD, BMI SDS. Statistical significance was defined as a two-sided $P<0.05$. Statistical analysis was performed using SAS (version 9.4, SAS Institute, Cary, NC, USA).

Results

Baseline characteristics

All the 95 patients (median age 13.5 years, range IQ 12.4-15.5 years) included in the study were affected by hypogonadism (35 with HH and 60 with POI). In 45/95 patients (47%) HPG impairment was a late effect of cancer treatments. Among them, 7 patients showed a condition of HH (CancerHH): 5 patients received cranial RT and surgery (n=4) or surgery alone (n=1) for craniopharyngioma treatment; one was treated with surgery and protontherapy for dysgerminoma and one with surgery and cranial RT for germinoma. The other 38 patients developed premature

ovarian insufficiency (CancerPOI) due to total body irradiation (TBI) and gonadotoxic chemotherapy for bone marrow transplant (n=35), craniospinal irradiation and gonadotoxic chemotherapy (n=3) for medulloblastoma, ependimoma and neuroblastoma.

In 22/95 patients (23%) HH was due to congenital isolated hypogonadotropic hypogonadism (CHH), with or without an underlining known genetic cause, whereas in 6/95 girls (6.5%) hypogonadism was associated with multiple pituitary hormone deficiencies (MPHD).

Moreover, in 15/95 patients (16%) ovarian failure was due to X chromosome abnormalities (confirmed by karyotype) in the spectrum of Turner syndrome (TS), whereas in 7/95 (7.5%) POI was secondary to monogenic, autoimmune or unknown origin (Supplementary material).

The baseline characteristics of whole cohort and of six groups according to the above-mentioned underlying diagnosis are shown in Table 1. It is worth mentioning that chronological age was significantly higher in patients with isolated CHH and POI (P<0.0001), height at baseline was significantly reduced in TS girls over the other groups (P<0.0001) and bone age was delayed in all patients, but with a lower BA-CA difference (P=0.001) and BA/CA ratio (P=0.001) in TS. No other difference was found between the six groups.

Moreover, 27 girls (28%) were concomitantly treated with rGH: 14/27 patients for short stature in TS, 13/27 for GHD secondary to late effects of cancer treatments (5/7 Cancer HH and 2/38 Cancer POI) or genetic/idiopathic cause (MPHD 6/6).

All the 49 girls who have completed pubertal induction showed a median age at start of 13.7 years (range IQ 12.5-15.6 years) and a mean uterine longitudinal diameter at baseline of 36 mm (range IQ 26 to 40 mm). As far as underlying diagnosis is concerned, patients were divided as

follows: CHH (n=12), isolated POI (n=4), TS (n=9), CancerHH (n=4), CancerPOI (n=17) and MPHD (n=3).

Induction regimen

All study patients started with low-dose TD 17β-oestradiol (median 0.14 mcg/kg/day, range IQ 0.1 to 0.2 mcg/kg/day) to be increased every six months (Fig.1a and 1b). As expected, serum estradiol levels followed the concomitant TD 17β-oestradiol increase (Fig.1c). In 16/95 (17%) induction was started with overnight-only TD 17β-oestradiol (with patches applied from 10:00 pm to 8:00 am). No association was found between oestradiol starting dosage and age at start, nor differences in starting dosage according to underling diagnosis.

As expected, oestradiol serum levels were positively correlated with Tanner stages (P<0.0001), with Tanner stage 1 always associated with concentrations under 40 ng/L (Fig.2a). In addition, TD 17β-oestradiol dosage at any point of assessment was directly associated with Tanner stages (P<0.0001), with Tanner stage 1 for 17β-oestradiol dosage under 0.2 mg/kg/day (Fig. 2b).

Moreover, serum oestradiol levels followed TD 17β-oestradiol increase with a direct association between the two variables at any point of assessment (P<0.0001, Fig. 3).

In 61/95 patients, progesterone (24 Dienogest, 27 Medroxyprogesterone acetate, 4 Micronized progesterone, 2 Norelgestromin, 2 Dihydroprogesterone, 1 Gestodene, 1 Nomegestrol acetate) was introduced at bleeding occurrence or at the achievement of uterine maturity according to clinical practice of the specific centre.

Out of 95 patients, 49 completed pubertal induction (2.65±1 years) with a mean of 2.63±0.97 number of dose changes before progesterone introduction (median 2.22 years, range IQ 1.56 to 2.87 years). Mean time at bleeding occurrence was 2.3±1 years.

Outcomes during induction

Auxological outcomes (either for the whole population or according to the six diagnostic categories) are listed in Table 2.

When considering the underlying diagnosis, no difference was found in mean time at B3, B4 and B5. As far as linear growth is concerned, the trend of height during ERT according to the underlying diagnosis is reported in Fig.4a. Oestrogen replacement therapy significantly reduced MPH distance (AH-MPH -0.68±1.56 SDS vs -1.37±1.37 SDS at baseline, $P<0.0001$). Nonetheless, CancerPOI and TS did not achieve their height potential, with AH SDS significantly lower than MPH SDS ($P=0.001$ and $P=0.03$, respectively) despite rGH concomitant treatment in 2/38 and 14/15 cases, respectively (Fig.4b). Patients with TS showed a reduced AH ($P<0.0001$), with a higher MPH distance ($P=0.001$) in respect to all the other girls.

Using a multivariate regression model, it was found that greater chronological age at baseline and underlying diagnosis were the main determinant of AH (SDS). Moreover, growth velocity during the first year of ERT showed a significant moderate negative correlation with baseline chronological age ($R=-0.59$, $P<0.0001$) and Tanner stage ($R=-0.41$, $P<0.0001$). The significant weight of confounding factors in our heterogeneous population did not allow us to conduct extrapolation of significant results on the effect of the characteristics of the therapy used. However, as far as AH SDS was concerned, there was a significant weak negative correlation between AH SDS and 17β-oestradiol (mcg/kg/day) at start ($R= -0.37$, $P=0.011$).

Outcomes at the end of the induction

Outcomes characteristics of the 49 patients with completed pubertal induction are shown in Table 3. Induction duration was not influenced by age at start and appeared to be not significantly different in the six groups.

Most girls (44/49, 90%) reached Tanner stage B4, but less than a half (20/49, 41%) Tanner stage B5. The achievement of the last Tanner stage was significantly associated with TD 17β-oestradiol dose at progesterone introduction and number of dose changes (P=0.034) even after correction for possible confounders (Tanner stage 1 or 2 at induction start, chronological age, BMI SDS at start and initial diagnosis).

Uterine data were available in 45/49 and 42/49 for longitudinal diameter and uterine shape, respectively.

Uterine longitudinal diameter showed a gradual increase during ERT (Fig.5a) with a significant association with oestradiol serum levels (R^2=0.2658, P<0.0001, Fig.5b) and TD 17β-oestradiol dosage (R^2=0.1249, P<0.0001) at any time of assessment (Fig.5c), but only in 17/45 (38%) cases longitudinal uterine diameter was longer than 65 mm (details about these 17 girls are available in Table 3). No association was found between ULD and age at start. As far as underlying diagnosis was concerned, ULD was significantly different in the six diagnostic groups (Table 3, One-way ANOVA), being reduced in CancerPOI vs others (P=0.001) despite no difference in the induction regimens used in the two groups (dose of 17β-oestradiol at start, at progesterone introduction, oestrogen total exposure, number of dose changes, induction time).

Despite a uterine longitudinal diameter below 65 mm, the majority of patients showed a uterus with adult shape (31/42, 74%), with a significant difference between those with hypogonadism

of genetic/idiopathic cause vs those with late effects of cancer treatment (P=0.001). Indeed, among the 11 girls who did not achieve a uterus with adult shape, 10 were CancerPOI. It is worth mentioning that among the 15 patients with POI secondary to late effects of cancer treatment 10 (67%) still showed a uterus with tubular (immature) shape (Table 3). Uterine maturation was directly associated with ULD length (P<0.0001).

When considering a multivariate model, having received TBI or pelvic RT was the major determinant of reduced ULD at the end of induction. After correction for uterine irradiation and other known clinical confounders, ULD was associated with 17β-oestradiol dose at progesterone introduction (Table 4). Uterine longitudinal diameter at the end of the induction was not significantly different from the one assessed at last follow-up after at least 6 months from COC introduction (P=0.544), indeed no difference was found in mean ULD assessed at last follow-up between patients receiving different type of progesterone (Dienogest vs other groups/Medrossiprogesterone acetate vs other groups).

Discussion

To the best of our knowledge, this represents the first retrospective longitudinal study on induction of puberty using TD 17β-oestradiol in a large cohort of hypogonadal females, including >50% of cancer survivors. Data on outcomes of pubertal induction have been so far collected from isolated experiences, small observational studies or clinical trials mostly on girls with TS, a population with clinical peculiarities in terms of treatment goals and risk factors. Actually, in our population, baseline characteristics as well as outcomes at the end of the induction were extremely different according to the underlying diagnosis, thus raising concerns on the generalization of information derived from studies performed in diagnostic categories which are not necessarily comparable (3).

At the aim of mimicking natural puberty, it is of paramount importance to consider two main outcomes, namely a gradual and complete breast development and an appropriate uterine maturation, given their crucial role in establishing self-esteem and in ensuring future opportunity of pregnancy.

The main results of the present study regard uterine maturation, which clearly mirrors the degree of estrogenization. It is well established that final uterine longitudinal diameter, though positive correlated with serum oestradiol levels, is usually significantly lower in girls with hypogonadism, despite ERT (4). Accordingly, considering the cohort who completed induction, the final ULD was suboptimal in more than a half of patients, with the worst outcomes in girls who received uterine irradiation (CancerPOI). The most important determinant of uterine maturation was history of cancer diagnosis and treatment, probably due to permanent changes that can negatively influence treatment response rather independently from the induction regimen used.

The TBI-based conditioning in patients attending bone marrow transplant can affect both ovaries and uterus, with a reduced uterine development, probably due to radiation-induced fibrosis and vasculature damage (32). A very recent prospective cohort study, has shown that uterine volume is diminished in patients who have received bone marrow transplant rather independently from the conditioning regimen received, further confirming our results. The underlying mechanism is far from being elucidated, nonetheless there are some suggestions that alkylating agents could induce uterine damage as well (32).

Consistently, after correction for CancerPOI, TD 17β-oestradiol dose at progesterone introduction was significantly associated to a better uterine response, thus suggesting that higher levels of TD 17β-oestradiol in the last part of the induction are required to optimize these

outcomes. The existence of a precise timing in uterine maturation is supported by the evidence that during pubertal development there is a significant correlation between uterine dimensions (30) and oestradiol concentration, with more striking changes at Tanner stage B3 and B4 (4; 33; 34; 35). Indeed, it has been hypothesized that there should be a critical window for uterine development during puberty, outside which reduced size cannot be recovered (3; 4). Our data demonstrate that no further uterine development occurs after progesterone introduction, with longitudinal diameter remaining unchanged after COC start. Hence, it leads us to wonder if introducing progesterone at bleeding occurrence is the right choice if mature breast (another important outcome of pubertal induction) and uterine development is still far to be completely achieved (4). Indeed, in our population the last Tanner stage has been achieved only in 40% cases, showing a significant association with gradual TD 17β-oestradiol incremental dosage and ERT dose at progesterone introduction.

Based on these important findings, performing a pelvic ultrasound before progesterone start should be considered as standard clinical practice. If signs of endometrial hyperplasia occur at pelvic US in the presence of underdeveloped breasts or uterus, then a slight reduction in 17β-oestradiol dose should be taken into consideration in order to delay the introduction of progestin, although this should be confirmed in studies on a larger scale with particular focus on CCS. Further underlying the need of an individualized therapeutic approach, the present results also suggest that increasing TD 17β-oestradiol dose before progestin introduction, with the aim to optimize uterine outcome, could be completely useless in some specific patients with scarce uterine response to ERT and history of bone marrow transplant and pelvic irradiation (32). At this regard, increasing the dosage can be not only useless but also potentially harmful in CCS,

who are “poor responder” to ERT but at higher risk of secondary neoplasms (even uterine cancer) per se (36).

In our cohort similar TD 17β-oestradiol doses and oestradiol serum levels corresponded to different Tanner stages, reflecting the heterogeneity of individual clinical response. However, Tanner stage I was always associated with TD 17β-oestradiol under 0.2 mcg/kg/day, thus reflecting the importance to maintain initial TD ERT under this dosage, in the attempt of reaching Tanner stage 3 after at least 12 months from induction start, either optimizing linear growth or avoiding poor breast development (usually with prominent nipple and scarce supporting breast tissue). Moreover, the dynamics of breast maturation are reported to be rather uniform, with achievement of stage B4 after approximately 2 years (37; 38), as confirmed in our cohort, with almost all girls achieving that stage before the end of the induction.

Puberty is characterized not only by secondary sexual characteristics development but also by a growth acceleration.

Basal auxological parameters further enlighten the differences between diagnostic categories: TS and CCS were shorter than other patients with a bone age not significantly delayed. As expected, this resulted in a worse AH, despite the reduction of MPH distance during treatment. Indeed, as already reported in literature, TS and CCS did not achieve their genetically-determined height potential, despite rGH concomitant treatment (39; 40). Conversely, CHH, POI and MPHD showed the highest AH, probably due to the underlying bone age delay at diagnosis and the long lasting pre-pubertal growth. Indeed, at baseline, girls with isolated CHH and POI were relatively older than others, because of the important diagnostic and treatment delay, reflecting the challenging differential diagnosis between HH and constitutional delay of growth and puberty (3; 41).

Some Authors suggest to start the pubertal induction with low dose TD 17β-oestradiol to be applied only at night (17) and then ERT should be gradually increased in order to mimic the spontaneous estrogenic levels in the early pubertal range as well as the diurnal pattern of serum 17β-oestradiol (12; 17; 42). The rationale of this approach is promoting gradual breast maturation as well as increasing growth velocity and "pubertal growth spurt". Up to now, no clinical study has demonstrated any actual superiority of one regimen versus the other. Our data show that low 17β-oestradiol dose at induction start, even considering overnight-only initial ERT, could be beneficial in terms of height gain in the first year of treatment and at AH. Nevertheless, our data are not conclusive due to the heterogeneity of the population, since age at induction and diagnosis appear to be strong confounders. It remains to be ascertained if the overnight ERT in some specific categories with important diagnostic delay, namely CHH and POI, prolonging pre-pubertal growth, should be reasonably skipped to avoid the development of eunucoid proportion, as abovementioned, and further delay in treatment.

The main limit of the present study is its retrospective nature, typical of data derived from clinical registry, however the rather uniform management strengthen the present results. It should also be acknowledged that, though preliminary data on a limited and heterogeneous cohort of patients, considering the low prevalence of congenital or acquired hypogonadism of pre-pubertal onset and the length of follow-up, the present sample size may be considered particularly relevant.

In conclusion, our data confirm the importance of starting pubertal induction with growth-promoting TD 17β-oestradiol dose, even considering initial overnight-only ERT, especially in patients with worse adult height outcomes in order to prevent premature epiphyseal fusion and optimize the eventual concomitant treatment with rGH. Present results also show the beneficial

effects of gradual ERT increase in terms of complete feminization and uterine development, firstly providing evidence of the importance of a correct and individualized timing of progesterone introduction, not merely based on breakthrough bleeding but on uterine dimensions and shape assessed at pelvic ultrasound, since progestins hamper the subsequent changes in uterine volume and the achievement of the last Tanner stage.

Due to the lack of licensed hormone preparations specific for pubertal induction, randomized-controlled studies are not feasible. Thus, collecting data through clinical registry represents an important strategy to optimize the management of these patients. Considering the peculiarity of our cohort, including patients with hypogonadism of different aetiologies, we will continue to collect data to confirm present results on a larger scale at the aim to tailor ERT at best.

Conflict of Interest

The authors have no conflict of interest to declare.

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Figure Legends

Fig.1 a. Oestradiol dose (mcg/kg/die); **b**. Oestradiol dose (mcg/die) **c**. Serum oestradiol levels at TD 17β-oestradiol increase.
Fig.2 a. Oestradiol serum levels and Tanner stages; **b**. Oestradiol dose (mcg/kg/die) and Tanner stages.
Fig.3 Serum oestradiol levels at TD 17β-oestradiol increase.
Fig.4 a. Height gain during induction according to initial diagnosis; **b**. Height gain at the end of induction and mid-parental height distance according to the initial diagnosis.
Fig.5 a. Uterine maturation during induction; **b**. correlation between uterine longitudinal diameter (ULD) and oestradiol levels at any time of assessment; **c**. correlation between ULD and TD 17β-oestradiol dose at any time of assessment.

Table 1. Baseline characteristics according to underlying diagnosis

Variable	All cohort (N=95)	Diagnosis						P-value
		CHH (N=22)	POI (N=7)	TS (N=15)	Cancer HH (N=7)	Cancer POI (N=38)	Pan HH (N=6)	
CA (years)	13.52	16.71	15.63	12.51	12.55	13.01	12.40	<.0001[b]
median [range IQ]	[12.40 to 15.53]	[16.04 to 17.89]	[15.22 to 16.38]	[11.88 to 13.87]	[11.97 to 12.63]	[12.35 to 14.04]	[11.66 to 13.16]	
MPH (cm)	164.39 (4.80)	166.56 (4.84)	164.80 (3.70)	163.80 (5.38)	159.93 (3.31)	163.80 (4.41)	164.75 (5.24)	0.065[a]
mean (SD)								
N. missing	*21*	*1*	*2*	*5*	*1*	*10*	*2*	
MPH (SDS)	0.18 (0.81)	0.55 (0.82)	0.26 (0.60)	0.09 (0.91)	-0.55 (0.55)	0.08 (0.74)	0.24 (0.88)	0.074[a]
mean (SD)								
N. missing	*21*	*1*	*2*	*5*	*1*	*10*	*2*	
HT baseline	149.11 (11.76)	161.50 (8.13)	161.67 (7.40)	136.94 (5.54)	141.01 (8.84)	145.76 (7.81)	150.13 (10.39)	<.0001[a]
mean (SD)								
HT (SDS)	-1.21 (1.37)	-0.15 (1.20)	0.08 (0.96)	-2.66 (0.73)	-1.86 (1.26)	-1.50 (0.89)	-0.42 (1.79)	<.0001[a]
mean (SD)								
Weight (kg)	44.10	56.90	49	36.80	48.40	40.40	47.40	0.0004[b]
median [range IQ]	[36.10 to 57.40]	[47 to 62.40]	[44.50 to 59.60]	[33 to 43.50]	[30 to 57.40]	[33 to 49.30]	[36.50 to 58]	
BMI (kg/m²)	19.41	20.04	18.67	19.07	22.89	18.72	20.14	0.573[b]
median [range IQ]	[17.34 to 23.81]	[18.49 to 23.09]	[17.38 to 23.19]	[17.89 to 22.99]	[18.70 to 26.53]	[16.23 to 24.97]	[18.36 to 25.35]	
BMI (SDS)	0.14 (1.47)	-0.16 (1.29)	-0.08 (1.54)	0.15 (1.10)	1.51 (1.75)	0.06 (1.52)	0.45 (1.92)	0.183[a]
mean (SD)								
Baseline serum estradiol (ng/L)	5	5	5	5	15.10	5	9.30	0.793[b]
median [range IQ]	[5 to 17]	[5 to 11.40]	[5 to 19]	[5 to 15.90]	[5 to 17.30]	[5 to 18.40]	[5 to 23.40]	
N. missing	*8*	*0*	*1*	*2*	*2*	*3*	*0*	
Longitudinal uterine diameter baseline (mm)	35	34	29	34.25	35	37	39	0.627[b]
median [range IQ]	[27 to 40]	[23 to 40]	[27 to 45]	[27 to 35]	[25 to 41.50]	[31 to 43]	[27 to 40]	
N. missing	*34*	*1*	*4*	*9*	*2*	*17*	*1*	
BA (years)	12	13	12.75	11	10	11.5	9.7	<.0001[b]
median [range IQ]	[10.25 to 13]	[12.10 to 14.50]	[12 to 13.85]	[10.25 to 12.75]	[7.90 to 10]	[10.50 to 12]	[8 to 10]	
N. missing	*18*	*4*	*3*	*3*	*0*	*7*	*1*	
BA - CA (years)	-1.73	-3.11	-2.85	-0.83	-2.63	-1.38	-2.92	0.0002[b]
median [range IQ]	[-2.96 to -0.78]	[-4.03 to -1.99]	[-3.90 to -1.72]	[-1.17 to -0.48]	[-3.79 to -1.76]	[-1.87 to -0.69]	[-2.99 to -2.67]	
N. missing	*18*	*4*	*3*	*3*	*0*	*7*	*1*	

[a] One-way ANOVA, [b] Kruskal-Wallis test. CA: chronological age; MPH: mid-parental height; HT: height; BMI: body mass index; BA: bone

Table 2. Auxological outcome according the underlying diagnosis

Variable	All cohort (N=95)	Diagnosis CHH (N=22)	POI (N=7)	TS (N=15)	CANCER HH (N=7)	CANCER POI (N=38)	MPHD (N=6)	P-value
ΔB3 (years)	1.20	1.36	0.92	1	1.42	1.07	1.79	0.462[b]
median [range IQ]	[0.79 to 1.64]	[0.87 to 1.66]	[0.66 to 1.33]	[0.66 to 1.48]	[0.46 to 1.65]	[0.73 to 1.72]	[1.01 to 2.11]	
N. missing	*9*	*1*	*0*	*0*	*1*	*6*	*1*	
ΔB4 (years)	2.05	2.01	1.81	1.66	2.61	2.12	2.80	0.034[b]
median [range IQ]	[1.61 to 2.63]	[1.52 to 2.34]	[1.64 to 1.94]	[1.46 to 2.44]	[2.40 to 3.31]	[1.71 to 2.97]	[2.53 to 2.99]	
N. missing	*31*	*6*	*2*	*4*	*2*	*16*	*1*	
ΔB5 (years)	3.13	2.95	2.65	4.44	5.31	4.01	-	0.108[b]
median [range IQ]	[2.55 to 4.10]	[2.51 to 3.30]	[2.51 to 2.79]	[3.39 to 6.25]	[3.43 to 7.19]	[2.43 to 4.50]		
N. missing	*73*	*12*	*4*	*11*	*5*	*35*	*6*	
AH (cm)	159.70 (10.25)	167.48 (7.55)	167.45 (7.12)	148.08 (6.47)	157.18 (6.58)	156.20 (8.24)	167 (10.64)	<.0001[a]
mean (SD)								
N. missing	*27*	*3*	*1*	*5*	*2*	*13*	*3*	
AH (SDS)	-0.58 (1.67)	0.68 (1.21)	0.68 (1.16)	-2.46 (1.07)	-1 (1.08)	-1.16 (1.35)	0.61 (1.74)	<.0001[a]
mean (SD)								
N. missing	*27*	*3*	*1*	*5*	*2*	*13*	*3*	
AH – MPH (cm)	-4.14 (9.59)	0.74 (8.09)	2.74 (7.65)	-13.80 (3.90)	-5.53 (5.15)	-9.36 (8.94)	4.17 (7.64)	0.0002[a]
mean (SD)								
N. missing	*43*	*4*	*2*	*9*	*3*	*22*	*3*	
AH – MPH (SDS)	-0.68 (1.56)	0.10 (1.32)	0.44 (1.26)	-2.22 (0.68)	-0.92 (0.88)	-1.53 (1.46)	0.69 (1.22)	0.0003[a]
mean (SD)								
N. missing	*43*	*4*	*2*	*9*	*3*	*22*	*3*	

[a] *One-way ANOVA,* [b] *Kruskal-Wallis test. B: Breast stage; AH: Adult height; MPH: Mid-parental height*

1 Table 3. Outcome characteristics

Variable	All cohort (N=49)	Diagnosis CHH (N=12)	POI (N=4)	TS (N=9)	CANCER HH (N=4)	CANCER POI (N=17)	MPHD (N=3)	P-value
Δ Induction (years)	2.65	2.53	2.76	2.70	3	2.75	2.51	0.968[c]
median [range IQ]	[1.72 to 3.20]	[1.72 to 3.24]	[2.08 to 3]	[1.65 to 2.84]	[2.23 to 3.90]	[1.77 to 3.31]	[2.18 to 4.64]	
B4 achievement								
N (%)								
No	5 (10%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	4 (24%)	0 (0%)	0.227[a]
Yes	44 (90%)	12 (100%)	3 (75%)	9 (100%)	4 (100%)	13 (76%)	3 (100%)	
B5 achievement								
N (%)								
No	29 (59%)	3 (25%)	2 (50%)	5 (56%)	2 (50%)	14 (82%)	3 (100%)	0.022[a]
Yes	20 (41%)	9 (75%)	2 (50%)	4 (44%)	2 (50%)	3 (18%)	0 (0%)	
Uterine length > 65 mm								
N (%)								
No	28 (57%)	7 (58%)	2 (50%)	4 (44%)	1 (25%)	13 (76%)	1 (33%)	0.263[a]
Yes	17 (35%)	5 (42%)	1 (25%)	3 (33%)	3 (75%)	3 (18%)	2 (67%)	
N. missing	*4*	*0*	*1*	*2*	*0*	*1*	*0*	
Uterine maturity								
N (%)								
No	11 (22%)	0 (0%)	0 (0%)	1 (11%)	0 (0%)	10 (59%)	0 (0%)	0.001[a]
Yes	31 (63%)	11 (92%)	3 (75%)	5 (56%)	4 (100%)	5 (29%)	3 (100%)	
N. missing	*7*	*1*	*1*	*3*	*0*	*2*	*0*	
Final uterine longitudinal diameter (mm)	61.22 (11.10)	63 (6.34)	58.33 (8.62)	65 (6.07)	70.5 (17.94)	54.47 (11.06)	70.83 (11.56)	0.027[b]
mean (SD)								
N. missing	*6*	*0*	*1*	*3*	*0*	*2*	*0*	
17β-estradiol dosage at progesterone introduction (mcg)	37.50	50	50	25	25	31.25	37.5	0.067[c]
median [range IQ]	[25 to 50]	[31.25 to 50]	[50 to 75]	[25 to 25]	[25 to 37.50]	[25 to 43.75]	[25 to 50]	
N. missing	*2*	*0*	*1*	*0*	*0*	*1*	*0*	
17β-estradiol dosage at progesterone introduction (mcg/kg)	0.70	0.79	1.10	0.61	0.58	0.67	0.51	0.319[c]
median [range IQ]	[0.50 to 0.86]	[0.50 to 0.80]	[1 to 1.13]	[0.58 to 0.80]	[0.51 to 0.93]	[0.50 to 0.92]	[0.34 to 1.25]	
N. missing	*2*	*0*	*1*	*0*	*0*	*1*	*0*	
17β-estradiol dosage at bleeding (mcg/die)	37.50	50	50	25	25	25	37.50	0.075[c]
median [range IQ]	[25 to 50]	[31.25 to 50]	[50 to 75]	[25 to 25]	[25 to 37.50]	[25 to 50]	[25 to 50]	

N. missing	*2*	*0*	*1*	*0*	*0*	*1*	*0*	
17β-estradiol dosage at bleeding (mcg/kg)	0.70	0.79	1.10	0.61	0.58	0.67	0.51	0.419[c]
median [range IQ]	[0.50 to 0.85]	[0.50 to 0.80]	[1 to 1.13]	[0.58 to 0.80]	[0.51 to 0.93]	[0.50 to 0.84]	[0.34 to 1.25]	
N. missing	*2*	*0*	*1*	*0*	*0*	*1*	*0*	

[a] *Fisher exact test,* [b] *One-way ANOVA,* [c] *Kruskal-Wallis test*

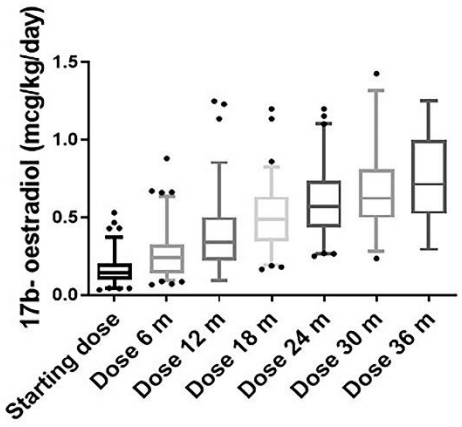
Table 4. Regression analysis

Linear regression uterine longitudinal diameter			
Variables	**β**	**CI**	**p-value**
Total estrogen exposure (mg)	0.101	(-0.219 to 0.422)	0.521
Mean time at Pg[1] introduction (years)	0.896	(-2.320 to 4.111)	0.571
Induction time (years)	0.895	(-2.390 to 4.180)	0.579
Dose 17βE_2[2]/24h intro Pg (µg)	0.232	(0.007 to 0.457)	0.043
Dose 17βE_2[2]/24h start (µg)	-0.345	(-0.978 to 0.287)	0.271
Number of increments ERT[3]	1.892	(-0.765 to 4.548)	0.155

*Confounders: CancerPOI (pelvic RT), Tanner stage at baseline, baseline longitudinal diameter, BMI SDS.
[1]Pg: progesterone
[2]E_2: estradiol
[3]ERT: estrogen replacement therapy

Fig.1

a.



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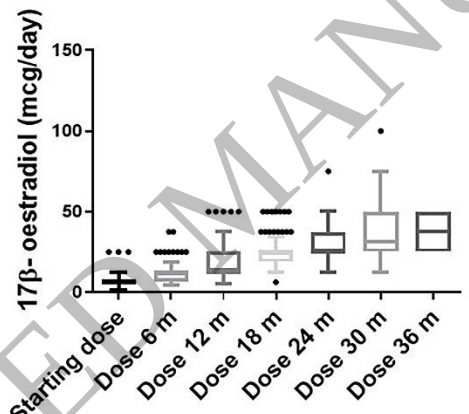

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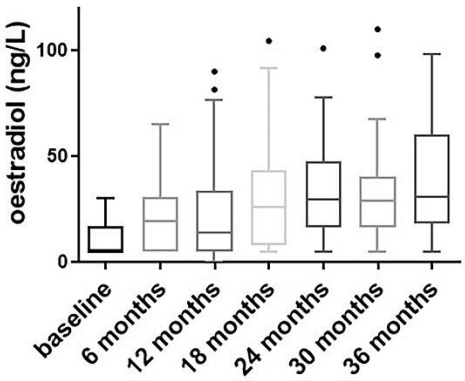

Figure 1
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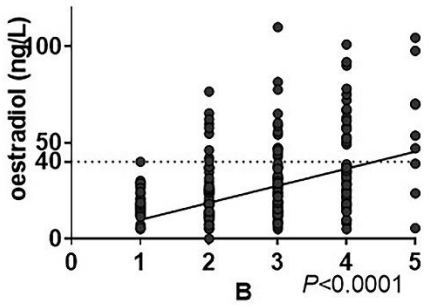
Fig.2

a.



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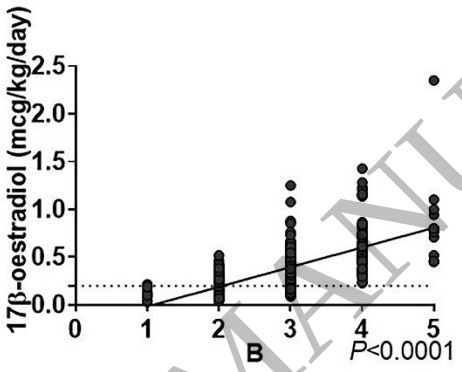


Figure 2
190x339 mm (x DPI)

Fig.3

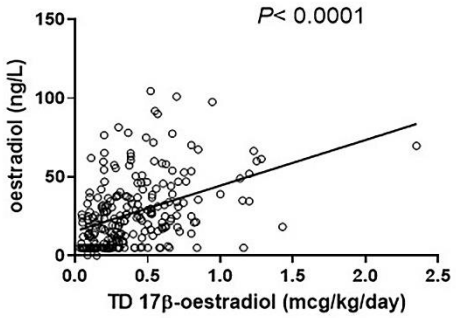
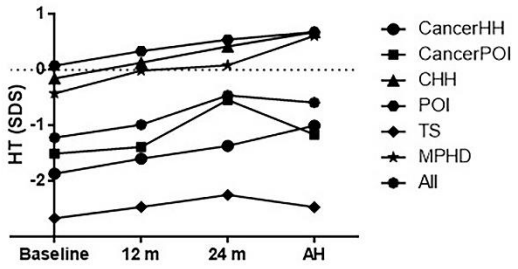


Figure 3
190x339 mm (x DPI)

Fig.4

a.



b.

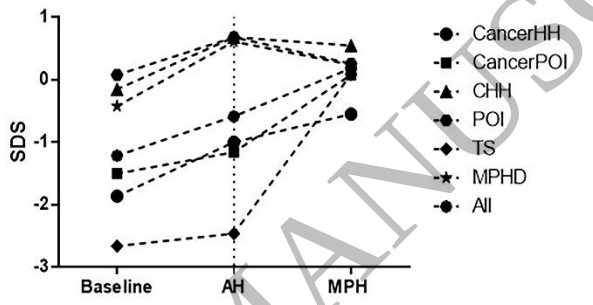
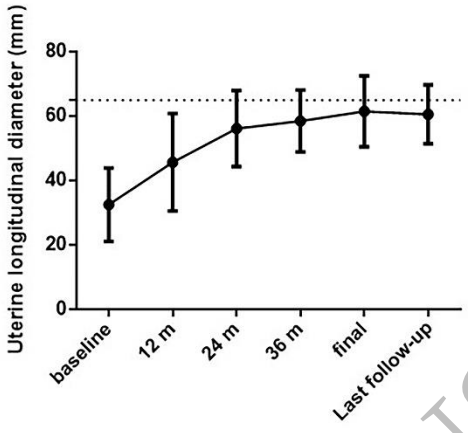


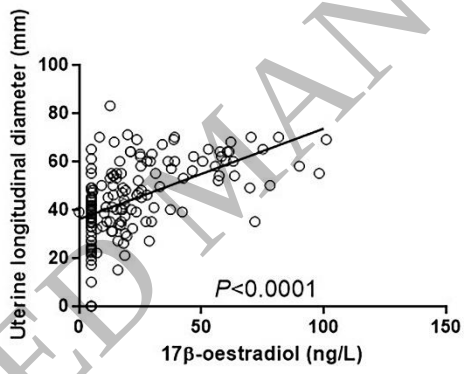
Figure 4
190x339 mm (x DPI)

Fig.5

a.



b.



c.

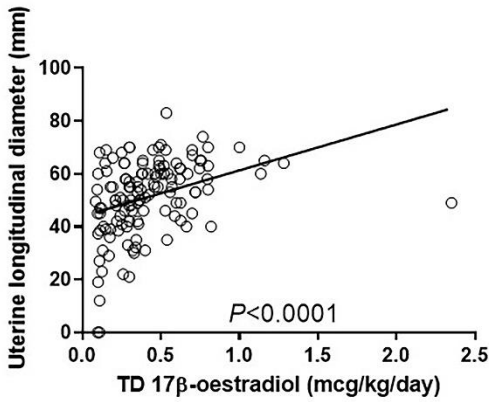


Figure 5
190x339 mm (x DPI)