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"Pubertal induction in girls with hypogonadism: estrogen replacement therapy outcomes and optimization of progesterone introduction"

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1. INTRODUCTION

1.1 Hypogonadism

1.1.1 Definition

The term "hypogonadism" refers to a pathological reduction of sex hormones production or reproductive activity of gonads. Hypogonadism can be either a consequence of a disorder of gonadal tissue, classified as primary hypogonadism (PH), or a defect within the hypothalamic-pituitary (HP) axis, resulting in a central hypogonadism (CH) (1). Hypogonadism is biochemically defined by low serum levels of the sex steroids (testosterone and oestradiol in men and women, respectively) being associated to variable serum levels of the gonadotrophins, according to the level of the defect (1).

Moreover, the underlying causes of hypogonadism can be congenital, as part of a genetic condition, or acquired (1).

The clinical manifestations of hypogonadism can be different according to the severity of the underlying defect and time of onset. In girls they include delayed puberty (defined as a retardation of pubertal onset beyond the 2-2.5 standard deviations of the expected age, namely 13 years for females), lack of pubertal development, primary or secondary amenorrhoea, anovulation as well as physical weakness, bone mineral loss, and, to some extent, emotional lability and reduced libido (Table 1, (1)). Moreover, in girls, PH is usually defined as primary ovarian insufficiency (POI), given the underlying driving role of the loss of ovarian function in the onset of the primary hypogonadism.

Table 1. Clinical manifestations of hypogonadism in females

Age of onset	Clinical manifestations
Foetal/neonatal	**No phenotype**
Minipuberty	**No phenotype**
Peri-pubertal	**Absent or incomplete puberty** **Eunuchoid proportions** **Primary amenorrhea**
Adult	**Secondary amenorrhea** **Low libido** **Infertility and anovulation** **Osteoporosis**

1.1.2 Hypogonadotropic Hypogonadism (HH)

Central Hypogonadism (CH), also known as hypogonadotropic hypogonadism (HH), can be due to all congenital and acquired causes that lead to a lack of gonadal stimulation by the HP axis and it is characterized by low levels of oestradiol with low/normal levels of gonadotropins (2).

There is no rigorous epidemiological study on the prevalence of HH in females, nonetheless examining the discharge registers of all five university hospitals in Finland a study estimated the prevalence of HH as 1 in 48.000 in the Finnish population, with a clear gender difference, being 1:30.000 in males and 1:125.000 in females, respectively (3). However, recent works suggest that the male:female ratio can be closer to 2:1 (4) or even equal (5) as partial HH may still be underdiagnosed in females due to subtle clinical presentation that resembles functional hypothalamic amenorrhea. Indeed, mild and nonsyndromic forms of HH can be frequently

treated with oral contraceptives or hormone replacement therapy (HRT) rather than receiving a complete diagnostic workup (6).

Congenital HH (CHH) is usually genetically inherited and a result of failure of embryonic migration of GnRH neurons into the hypothalamus or their functional impairment.

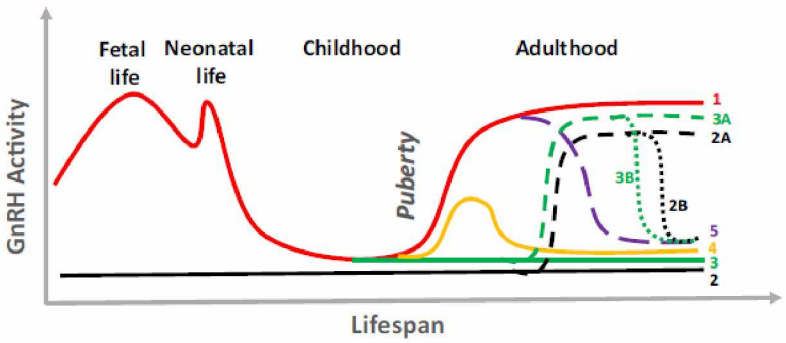
Several mutations have been identified as causally related to HH and they can be grouped in three main categories:

(i) mutations in genes associated with neuronal differentiation and migration;

(ii) genes involved in GnRH secretion;

(iii) genes related to the development of GnRH receptors.

Each gene mutation has a variable effect on GnRH neuronal migration or functioning that in turn determines the severity of the clinical phenotype. This ranges from the absence of mini-puberty during the first 3-6 months of life, mild constitutional delay of puberty to more severe phenotypes (Fig.1) (2; 7).



1: normal GnRH secretion profile
2 and 3: complete defect of GnRH secretion
2A & 3A: reseversal of GnRH secretion
2B & 3B: relapse of GnRH secretion defect
4: partial defect of GnRH secretion
5: adult-onset GnRH secretion defect

Moreover, there is a wide heterogeneity in the transmission of gene mutations for CHH that include autosomal dominant, autosomal recessive and X-linked inheritance. Nonetheless, the majority of cases are sporadic, occurring in the absence of any family history.

The shared origin of olfactory and GnRH secretory neurons provides an explanation for the association of CHH with anosmia or hyposmia in around 50% of cases of HH (2).

However, CHH, even if initially divided into Kallmann Syndrome (KS) with olfactory dysfunction (anosmia or hyposmia) and normosmic HH (NHH), is currently considered as a single entity, given the common genetic origin of KS and NHH. In addition to anosmia or hyposmia, patients with CHH may also present associated with other developmental anomalies such as midline defects (i.e. cleft lip or palate), dental agenesis, ear abnormalities, sensorineural deficits, renal agenesis, bimanual synkinesis or skeletal anomalies (7).

In the absence of any of the aforementioned signs (usually considered as "red flags" for CHH), prepubertal onset of CHH is clinically indistinguishable from the more frequent entity of constitutional delay of growth and puberty (CDGP), which is often considered as an extreme variant of normal pubertal timing characterized by a transient GnRH deficiency with spontaneous resolution (6). Despite being initially identical from the clinical point of view, differentiating CHH from CDGP is extremely important to avoid delay in HRT start (6). Another important differential diagnosis of CHH is functional hypothalamic amenorrhea, being the two conditions biochemically indistinguishable. Functional amenorrhoea refers to a chronic condition of anovulation in the absence of an underlying organic cause and in the presence of environmental triggers, i.e., undernutrition, eating disorders, stress or overtraining (8). In the diagnostic work-up of hypothalamic amenorrhoea the collection of anamnestic data plays a crucial role (helping in identifying risk behaviours) as well as the assessment of possible systemic and endocrinologic aetiologies, being ultimately a diagnosis of exclusion. Even in this context, differentiating functional hypogonadism is important, being usually reversible after life-style interventions (9).

The most frequent mutations associated with CHH are listed in Table 2 (7).

Table 2. Genes implicated in CHH

Gene	OMIM	CTO	CHH phenotypes			Overlapping syndromes									
			KS	CHH	CHH reversal	CPHD	CPHD + SOD	WS	CHARGE	HS	SHFM	D-WS	MGS	PEPNS	GHS
KAL1 (ANOS1)	300836	✓	✓	×	✓	×	×	×	×	×	×	×	×	×	×
SEMA3A	614897	✓	✓	×	×	×	×	×	×	×	×	×	×	×	×
SOX10	602229	×	✓	×	×	×	×	✓	×	×	×	×	×	×	×
OL14RD	606807	✓	✓	×	×	×	×	×	×	×	×	×	×	×	×
HESX1	182230	×	✓	×	×	✓	✓	×	×	×	×	×	×	×	×
FEZF1	613301	×	✓	×	×	×	×	×	×	×	×	×	×	×	×
FGFR1	147950	✓	✓	✓	✓	✓	✓	×	×	✓	✓	×	×	×	×
FGF8	612702	✓	✓	✓	×	✓	×	×	×	×	×	×	×	×	×
CHD7	612370	×	✓	✓	✓	×	×	×	✓	×	×	×	×	×	×
FGF17	603725	✓	✓	✓	×	×	×	×	×	×	×	✓	×	×	×
HS6ST1	614880	✓	✓	✓	✓	×	×	×	×	×	×	×	×	×	×
PROK2	610628	✓	✓	✓	×	×	×	×	×	×	×	×	×	×	×
PROKR2	147950	✓	✓	✓	✓	✓	×	×	×	×	×	×	✓	×	×
SEMA7A	607961	✓	✓	✓	×	×	×	×	×	×	×	×	×	×	×
WDR11	614858	✓	✓	✓	×	✓	×	×	×	×	×	×	×	×	×
NSMF	614838	✓	✓	✓	✓	×	×	×	×	×	×	×	×	×	×
AXL	109135	×	✓	✓	×	×	×	×	×	×	×	×	×	×	×
GNRH1	614841	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
GNRHR	146110	✓	×	✓	✓	×	×	×	×	×	×	×	×	×	×
KISS1	614842	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
KISS1R	614837	✓	×	✓	×	×	×	×	×	×	×	×	×	×	×
TAC3	614839	✓	×	✓	✓	×	×	×	×	×	×	×	×	×	×
TACR3	614840	✓	×	✓	✓	×	×	×	×	×	×	×	×	×	×
LEP	614962	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
LEPR	614963	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
PCSK1	162150	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
DMXL2	616113	×	×	✓	×	×	×	×	×	×	×	×	×	✓	×
RNF216	609948	×	×	✓	×	×	×	×	×	×	×	×	×	×	✓
OTUD4	611744	×	×	✓	×	×	×	×	×	×	×	×	×	×	✓
PNPLA6	603197	×	×	✓	×	×	×	×	×	×	×	×	×	×	✓
NR0B1	300200	×	×	✓	×	×	×	×	×	×	×	×	×	×	×

Abbreviations: CHH, congenital hypogonadotropic hypogonadism; CHARGE, coloboma, heart defects, atresia of choanae, retardation of growth and/or development, genital and/or urinary defects, ear anomalies or deafness; CPHD, combined pituitary hormone deficiency; CTO, contributes to oligogenicity; D-WS, Dandy-Walker syndrome; GHS, Gordon Holmes syndrome; HS, Hartsfield syndrome; KS, Kallmann syndrome; MGS, Morning Glory syndrome; OMIN, online Mendelian inheritance in man; PEPNS, polyendocrine deficiencies and polyneuropathies; SHFM, split-hand/foot malformation; SOD, septo-optic dysplasia; WS, Waardenburg syndrome.

Acquired causes of HH mostly develop before, during or after puberty, and can result from any type of pituitary or hypothalamic lesion (including craniopharyngiomas and HP tumours), and/or iatrogenically from the treatments of central nervous system (CNS) lesions (including surgical resection and radiotherapy-RT) (1; 10). Further acquired causes of HH include pituitary trauma, the use of exogenous steroids, pituitary iron overload from hereditary haemochromatosis or beta-thalassemia, and infiltrative conditions including hypothalamic disorders (11; 12). Ultimately, these conditions of acquired HH impair the function of gonadotroph cells within the anterior pituitary and/or reduce the function of gonadotropin

releasing hormone (GnRH) neurons, and thereby reduce the release of FSH and LH with consequent absence of menstruation and sub-infertility in females (13).

The severity of HH is usually commensurate with the severity of the underlying condition (14). Conversely, HH patients can revert, permanently or transiently, to a normal autonomous function of the reproductive axis (15; 16)(Fig. 1 (2)). This phenomenon, known as reversal, is not uncommon even in patients with underlying genetic HH, with a prevalence among CHH patients ranging from 5–10% (15; 16) to 20% (17). Even though mutations in TAC3 and TACR3 genes may particularly predispose to reversal (18; 19), no reliable genotype–phenotype correlation has been found with possibility of reversal also in CHH patients with different mutations. However, all data available in literature about reversal in HH are related to males, no convincing cases of this phenomenon have been documented in females, possibly due to the important clinical overlap with hypothalamic amenorrhea, which is far more common and wherein periods of remission and relapse over time are characteristic (2). Nonetheless, as well as in male counterparts and patients with POI, even in the absence of reliable prevalence data, relapsing in CHH or acquired HH is likely to occur even in females and should be taken into consideration in the setting of a long-lasting HRT.

1.1.3 Primary ovarian insufficiency

Primary ovarian insufficiency (POI) is characterized by a loss of ovarian function before the age of 40 accounting for one of the major causes of female infertility. Primary ovarian insufficiency is highly heterogeneous: the most severe forms present with absent pubertal development and primary amenorrhoea (50% of these cases due to ovarian dysgenesis), whereas forms with post-pubertal onset are characterized by disappearance of menstrual cycles (secondary amenorrhea) associated with premature follicular depletion.

Primary ovarian insufficiency is biochemically defined by low levels of gonadal hormones (estrogens and inhibins) and high levels of gonadotropins. This condition can be due to congenital or acquired causes, nonetheless, in most cases, POI remains classified as idiopathic (13).

Beyond infertility, hormone defects may cause severe neurological, metabolic or cardiovascular consequences and lead to the early onset of osteoporosis. Heterogeneity of POI is also reflected by the variety of possible causes, including autoimmunity, toxics, drugs, as well as genetic defects.

As far as the congenital origins are concerned, POI has a strong genetic component. In this context, X chromosome abnormalities in the spectrum of Turner Syndrome (TS) represent the major cause of primary amenorrhoea associated with ovarian dysgenesis.

Turner syndrome is a rare condition associated with complete or partial loss of one X chromosome, often in mosaic karyotypes, with a reported prevalence of ~50 per 100.000 women in different ethnic populations (20; 21). The condition is accompanied by primary hypogonadism in almost all patients, leading to either primary or secondary amenorrhoea and ultimately to infertility, with only few women retaining spontaneous fertility (21). The exact mechanisms behind POI in TS is still unknown, however, the accelerated loss of oocytes from the ovaries is probably early onset as already present during fetal life (22). Nonetheless, TS is a complex condition in which POI represents an important but not the only clinical aspect, being associated to variable grade of growth impairment and short stature, congenital heart defects, higher risk of type 1 and type 2 diabetes mellitus, osteoporosis and autoimmune disorders. All the clinical manifestations of TS are reported in Table 3 (21).

Table 3. Clinical manifestations of Turner Syndrome and their reported prevalence

Endocrine disorders
- Growth failure and reduced adult height (95–100%)
- Hypergonadotropic hypogonadism (90–95%)
- Glucose intolerance (15–50%)
- Type 1 diabetes mellitus (unknown frequency)
- Type 2 diabetes mellitus (10%)
- Thyreoiditis and hypothyreosis (15–30)
- Android body composition (unknown frequency)
- Autoimmune diseases (increased risk of all autoimmune conditions)

Gastrointestinal and hepatic disorders
- Elevated hepatic enzymes (50–80%)
- Coeliac disease (8%)
- Inflammatory bowel disease (2–3%)

Phenotypic characteristics: eyes
- Epicanthus (20%)
- Nearsightedness (20%)
- Strabismus (15%)
- Ptosis (10%)

Phenotypic characteristics: ears
- Infections of the middle ear (60%)
- Hearing defects (30%)
- Deformity of external ear (15%)

Phenotypic characteristics: mouth
- Micrognathia, defined as small mandibular bone (60%)
- High-arched palate (35%)
- Abnormal dental development (unknown frequency)

Phenotypic characteristics: neck
- Low posterior hairline (40%)
- Broad short-appearing neck (40%)
- Pterygium colli, defined as webbed neck (40%)

Phenotypic characteristics: thorax
- Broad chest, defined as shield chest (30%)
- Inverted nipples (5%)

Phenotypic characteristics: skin, nails and hair
- Increased skin ridge count (30%)
- Lymphedema of hands and feet (25%)
- Multiple pigmented naevi (25%)
- Nail hypoplasia/dystrophy (10%)
- Vitiligo (5%)
- Alopecia (5%)

Phenotypic characteristics: skeleton
- Bone age delay (85%)
- Decreased bone mineral content[a] (50–80%)
- Cubitus valgus (50%)
- Short fourth metacarpal (35%)
- Genu valgum (35%)
- Congenital hip luxation (20%)
- Scoliosis (10%)
- Madelung deformity (5%)

Phenotypic characteristics: heart
- Bicuspid aortic valve (14–34%)
- Coarctation of the aorta (7–14%)
- Aortic dilation/aneurysm (3–42%)
- Hypertension (50%)

Phenotypic characteristics: kidneys
- Horseshoe kidney (10%)
- Abnormal positioning or duplication of renal pelvis, ureters or vessels (15%)
- Renal aplasia (3%)

Neurocognitive and psychosocial issues[b]
- Emotional immaturity (~40%)
- Specific (nonverbal) learning disorder (~40%)
- Psychological and behavioral problems (~25%)
- Failure to thrive during first year of life (50%)

Data from REFS[1,3,4,34]. Please note that the approximate prevalence may differ between different karyotypic groups and, in general, women with the 45,X karyotype are more affected than other karyotypic groups. [a]In the absence of appropriate hormonal substitution therapy. [b]The data are inconsistent, and the given percentages should be viewed with caution.

Even though TS accounts for the vast majority of the genetic causes of POI, especially when early onset, sometimes it can be due to monogenic mutations. Nonetheless, the extreme variability in expressivity of POI, even in the same family, may indicate its multifactorial or oligogenic origin. Despite the description of several candidate genes, the cause of POI remains often undetermined (23).

The most frequent mutations associated with POI are listed in Table 4 (23).

Table 4. Genetic causes of premature ovarian insufficiency

	Estimated frequency in POI	References
X chromosome defects		
Turner's syndrome and related defects	4–5%	(9, 55, 59, 60)
Triple X syndrome	1–4%	(62)
Fragile X syndrome (*FMR1* premutation)	3–15%	(119, 120, 122, 132)
DIAPH2 disruption (translocation)	Unknown	(18, 19)
BMP15 variants	1.5–12%	(143–149)
PGRMC1 variants	1.5%	(155)
Autosomal defects		
Complex diseases	Rare	
Galactosemia (*GALT*)		(94, 95, 98)
BPES (*FOXL2*)		(79, 88)
APECED (*AIRE*)		(65, 70, 71)
Mitochondrial diseases (*POLG*)		(102, 103)
Demirhan syndrome (*BMPR1B*)		(109)
PHP1a (*GNAS*)		(101)
Ovarioleucodystrophy (*EIF2B*)		(106)
Ataxia telangiectasia (*ATM*)		(108)
Perrault syndrome (*HSD17B4*, *HARS2*, *CLPP*, *LARS2*, *C10ORF2*)		[a]
Premature aging syndromes:		
Bloom syndrome (*BLM*)		(113, 114)
Werner syndrome (*WRN*)		(117)
GAPO disease (*ANTXR1*)		(118)
Isolated disease		
FSH/LH resistance (*FSHR* and *LHCGR*)	0–1%	(42, 47, 134, 135)
INHA variants	0–11%	(138, 139)
GDF9 variants	1.4%	(32, 146, 150)
FOXL2 variants	Rare	(50, 92, 93)
FOXO3 variants	2.2%	(168, 169)
NOBOX variants	0–6%	(50, 175, 177–179, 182)
FIGLA variants	1–2%[b]	(183, 184)
NR5A1 variants	1.6%	(164, 165)
LHX8 variants	Rare	(50, 185, 186)
DNA replication/meiosis and DNA repair genes variants (*DMC1*, *MSH4*, *MSH5*, *SPO11*, *STAG3*, *SMC1β*, *REC8*, *POF1B*, *HFM1*, *MCM8*, *MCM9*, *SYCE1*, *PSMC3IP*, *NUP107*, *FANCA*, *FANCC*,*FANCG*)	Unknown	(35, 38–41, 45, 156–162)

FSH, follicle-stimulating hormone; LH, luteinizing hormone; POI, primary ovarian insufficiency.
[a]Refer to specific article on this same issue.
[b]1% in Indian and 2% in Chinese women, respectively.

Acquired forms, such as those occurring after cancer treatments or autoimmune diseases, account for many cases of POI as well.

Autoimmune causes can be usually included in the spectrum of the polyglandular autoimmune syndromes, as well as autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy (APECED) due to mutations in AIRE gene. In this context, in the diagnostic process of POI, in the absence of a known underlying genetic mutation or other acquired causes, autoimmune screening is mandatory (13).

1.1.4 Hypogonadism in Childhood Cancer Survivors (CCS)

Over the last decades, survival rates of childhood and adolescent cancer have steadily increased thanks to the improvements in the knowledge of the underlying molecular biology, the refinement of the diagnostic procedures and the discovery of novel treatment strategies (24; 25). Thus, nowadays a great proportion of patients, between 0.1 and 0.5% of the Western Countries population, have received cytostatic drugs or radiation or a combination of the two for childhood cancer (26; 27). Therefore, the burden of late effects of neoplastic treatments represents a new challenge in care management of these patients (28; 29; 30). Indeed, up to 75% of childhood cancer survivors (CCS) experience at least one iatrogenic effect in the long-term (31).

In this setting, endocrine sequelae are one of the most frequently observed: one of two CCS, indeed, will develop at least one endocrine complication during her lifespan as a late-effect of radiotherapy and alkylating agent chemotherapy.

Among the endocrine late-effects, hypogonadism is a frequent finding after antineoplastic treatment, being either a result of gonadal failure (POI) or a consequence of impaired gonadotropin secretion due to hypothalamic-pituitary dysfunction (HH) (32).

In CCS, HH has a reported prevalence of 6.5% overall (33) and 11% among those exposed to cranial radiotherapy (34). The main risk factors are tumor growth, surgery and radiation at doses ≥30 Gy affecting the hypothalamic-pituitary region (34; 35). Less frequently, HH could also result as a late-effect of the hypothalamic-pituitary irradiation at lower doses with longer follow-up (34), total body irradiation (TBI) (33), as well as those treated with radiotherapy for non-brain solid tumors of the head (34) and hypophysitis due to the use of anti CTLA-4 antibody (i.e. ipilimumab) (36).

As far as POI is concerned, the gonadal function is vulnerable to gonadotoxic chemotherapy (i.e. alkylating agents, Table 5 (37)) and radiotherapy (38).

Table 5. Anti-neoplastic drugs associated to gonadal toxicity

Most commonly used drugs by category
Alkylating agents and non-classical alkylators
Busulfan
Carmustine (BCNU)
Chlorambucil
Cyclophosphamide
Dacarbazine
Ifosfamide
Lomustine (CCNU)
Mechlorethamine
Melphalan
Procarbazine
Temozolomide
Thiotepa
Heavy metals
Carboplatin
Cisplatin

As most patients undergo polypharmacological treatment regimens, it is difficult to understand the relative risk related to each agent. However, the likelihood of developing POI is dose-dependent, thus, in order to estimate the cumulative detrimental effect of each agent, the cyclophosphamide equivalent dose (CED) method has been introduced (calculated according to the equation reported in Appendix A) (39). According to these mathematical models, a CED lower than 4000 mg/sm indicates an overall low risk of POI, but there is a progressive increase of female risk of infertility for incremental doses with the probability of having a pregnancy dropping below 50% for CED > 8000 mg/sm (40; 31). Moreover, radiation-induced ovarian damage has been proved to be dose-dependent as well. Radiotherapy is rarely administered alone, thus defining a sterilizing threshold could be extremely difficult. However, according to data obtained through the oocyte decline rate, abdominal radiation doses above 20.3 Gy in infants, 18.4 Gy at the age of 10 years, and 16.5 Gy at the age of 20 years are associated with high risk of POI (41; 40).

Moreover, there is a strong interdependence between oocyte and hormone-producing granulosa cells in the ovarian follicle (38). Indeed, the functional dichotomy (with distinct endocrine/reproductive compartments) typical of testes is absent in the ovary. In this context, POI is always associated with estrogen deficiency and fertility impairment (37).

Among female CCS exposed to high-risk chemo- and radiotherapy, the prevalence of POI is around 11.8% (42) but it can be as higher as 25.8% in females surviving medulloblastoma (43) or form 44% to 100% in female CCS conditioned for hematopoietic stem-cell transplant (HSCT) (44; 45).

HSCT, indeed, is becoming the standard-of-care for children affected by severe non-oncological conditions, such as primary immunodeficiencies, hemoglobinopathies and inborn errors of metabolism, even though malignancies still represent the most frequent indication for HSCT. In this setting, the conditioning regimen received before HSCT mainly affects the ovarian reserve per se. Furthermore, type of conditioning regimen, age and pubertal status at the time of HSCT also play a pivotal role on gonadal function: for instance, roughly 50% of female CCS treated with TBI under the age of 10 can spontaneously complete pubertal development, while nearly all the patients receiving treatment later in childhood experience POI (37). Although younger age and pre-pubertal status are protective factors, the risk of a late-onset POI remains high after TBI (37). Moreover, in patients undergoing chemotherapy-only based conditioning regimens, POI has been reported with rates as high as 100% after standard-dose busulfan (46; 47) as well as cyclophosphamide or melphalan alone (48; 49).

Hypogonadotropic hypogonadism, POI or even mixed forms of gonadal dysfunction (in patients who received both hypothalamic-pituitary and gonadal damage) in CCS can present with pubertal delay or absence of pubertal development, arrested puberty, primary or secondary amenorrhoea and sex hormone deprivation symptoms (37).

Moreover, the management of gonadal failure in CCS should take into consideration the associated clinical features of cancer survivorship, as listed in Table 6 (40).

Table 6. Specific clinical features of Childhood Cancer Survivors (CCS)

Increased incidence of metabolic syndrome
Higher risk of low bone mineral density – deficient accrual of bone mineral peak
Incomplete development of uterine volume in case of pelvic irradiation
Higher incidence of liver focal nodular hyperplasia and chronic iatrogenic hepatic toxicity
Growth impairment secondary to iatrogenic growth hormone deficiency or due to direct iatrogenic effect of radiation on growth plates
Higher incidence of hypoandrogenism
Chronic skin GvHD, both involving deficient bioavailability of hormones delivered transdermally and increasing risk of poor tolerance to transdermal patches
Vulvovaginal GvHD

1.2 Pubertal Induction

The increasing knowledge in the field of inherited and acquired disorders of the hypothalamic-pituitary-gonadal axis as well as the increased survival rate of chronic diseases leads to a new need to focus on adult bone and hormonal health. Most children with chronic diseases, indeed, now survive through adulthood, and may require pubertal induction and long-term HRT (50). Moreover, in the last 25-30 years, the overall 5-years survival rate for children and adolescents with cancer have remarkably increased reaching about 70-80%, due to the substantial improvements in the comprehension of cancer molecular biology, refinement of diagnostic techniques and novel therapeutic strategies (24; 25).

As aforementioned, in the presence of an early onset hypothalamic-pituitary-gonadal axis impairment, pubertal induction with HRT is mandatory.

The aim of pubertal induction in girls with hypogonadism is to resemble natural puberty, in terms of physical and psychological development. Puberty represents a physical milestone in life and a prerequisite for a normal adult life. The average duration of physiological pubertal development is between 4 and 4.5 years in healthy subjects, the time in which a girl undergoes through all the five Tanner stages, from I (pre-pubertal) to V (complete puberty) (51). Indeed,

the initial breast development, designated as change from Tanner stage B1 to B2 (breast budding), is considered the first sign of puberty in girls (52) and it is probably driven by the GnRH-induced pulses of LH, first occurring overnight (6). On the contrary, pubarche is usually not regarded as a marker for pubertal onset as it may be independent of HPG axis activation, resulting from maturation of the adrenal glands (adrenarche) (53).

The induction of puberty in hypogonadal girls needs to be carefully performed to assure an adequate growth spurt, a final 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 (50; 53).

The optimal estro-progestin (EP) replacement therapy regimen to induce puberty and maintain beneficial effects in adults is still being studied.

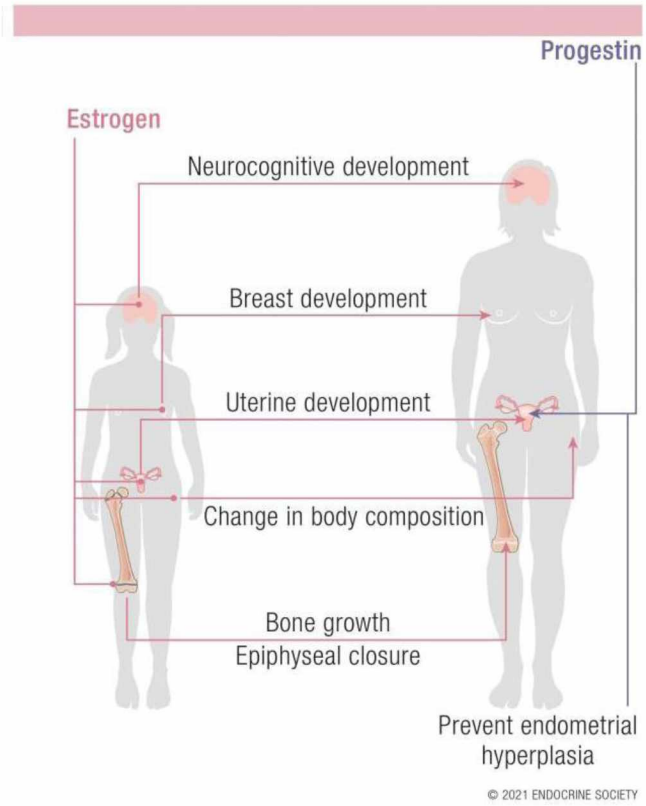
An integrated approach to hormonal management of acquired illnesses of childhood that affect pubertal timing and progress is required to optimize outcomes. Moreover, pubertal induction optimization with HRT should aim at offering an eventual future opportunity of conception that was not even considered possible in the past (50).

1.2.1 Estrogens

1.2.1.1 Phases of Pubertal Induction

In girls, adequate maturation of secondary sex characteristics is achieved with estrogen alone, whereas progesterone rises only in the late stage of puberty to prevent endometrial hyperplasia (Fig.2) (54; 55).

Fig.2 Role of estrogen and progestin



Thus, induction of puberty should start with low dose estrogen replacement to be gradually increased to achieve the complete pubertal development, taking into consideration that the process can be divided into two different parts. The first part, characterized by low estrogen doses, is crucial to promote the onset of pubertal growth and a physiological breast development; the second part, with increased estrogen doses, is fundamental to finalize bone mass accrual and uterine maturation (56). Moreover, the optimal estrogen treatment route, drug, dose and dosing tempo should be personalized (57).

The gradual increase of the low-dose estrogen replacement typical of the first part of the induction is important to allow longitudinal growth (pubertal growth spurt), to prevent premature epiphyseal fusion and to guarantee an adequate breast development, reaching mid-puberty (Tanner stage 3) after 15-24 months after start (58). High dose estrogen during early

puberty, indeed, may result in a reduced adult height and a poor breast development (usually with prominent nipple development and scarce supporting breast tissue) (59).

Nonetheless, in the second part, oestradiol concentrations should be increased in order to assure and appropriate bone mass accrual and uterine development (60; 61). Indeed, puberty represents a crucial time to optimize peak bone mass achievement, a fundamental determinant of future fracture risk (61).

In addition, one of the most important effects of estrogen is to promote uterine development for future conception. Indeed, during ERT, the uterus gradually changes from a tubular structure into a heart-shaped mature configuration increasing in size in all dimensions (62). Even though some uterine growth is found even in patients of 6 years of age, the most part of uterine development occurs during puberty and sometimes lasts after menarche. In the absence of an appropriate estrogen exposure, the uterus remains hypoplastic or even apparently absent at pelvic ultrasound. Moreover, in CCS previously treated with pelvic radiotherapy, the insufficient estrogen exposure is further aggravated by the known detrimental effect of irradiation on uterine maturation (63). To date, reproductive technology allows women with HH and even some with POI to achieve parenthood, the latter with fertility preservation or egg donation. In this context, poor uterine development (immature uterine development, poor endometrial thickness and deficient uterine vascularity) represents one of the contributing factors for negative pregnancy outcomes of this specific population (62; 63; 64; 65)

Puberty is a crucial period not only for feminization, but also for adequate peak bone mass achievement, which represents one of the most important determinants of adult fracture risk and cannot be reached beyond the age of 20-25 years. The 40-50% of total bone mass, indeed, is gained during puberty. Cortical bone accrual and trabecular mineralization parallel the linear growth curve with peak bone mass achievement by the end of adolescence. In this context, it is extremely important to address bone health as a key feature in paediatric endocrine

management, especially in adolescents with pubertal failure or arrest as long as poor nutrition or immobilization. Despite the acknowledgement that puberty usually provides the greatest increase in bone mass and largest lifetime reduction in fracture risk (66; 67), the place of pubertal treatment in bone health has only been recently addressed. Indeed, excessive delay in puberty and/or in pubertal induction may adversely affect bone health, although the data are not entirely consistent. Despite the only studies present in literature have addressed this condition especially in males (68; 69; 70; 71), pubertal delay can lead to a lower peak bone mass in girls, as well (72). Moreover, as far as CHH is concerned, De Rosa and colleagues (73) found an inverse correlation between spinal BMD and the age at pubertal induction, thus supporting the importance of early diagnosis and intervention. Indeed, it is well ascertained that histories of deficient treatment (inadequate dosage and/or long pauses) after diagnosis are also associated with reduced bone mass (74).

1.2.1.2 Type of estrogen and route of administration

To date, several studies in literature support the effectiveness and theoretical benefits of starting pubertal ERT with low-dose 17β-estradiol, being more physiological than ethinylestradiol (EE, a very potent synthetic analogue that is not metabolized to oestradiol). Indeed, it is a precautionary principle to replace any given endocrine deficiency with the bioidentical hormone, whenever possible (59; 60; 75; 76). Moreover, data from hypopituitary females receiving combined estrogen and GH treatment indicate a markedly greater impairment of GH-mediated insulin-like growth factor 1 (IGF-I) synthesis with EE than with 17β-estradiol (77), with a smaller inhibitory effect observed by taking transdermal vs oral 17β-estradiol (78): an aspect to take into account in pubertal girls who are expected to show an appropriate pubertal growth spurt or in patients under concomitant recombinant growth hormone (rGH) therapy. In this context, theoretically, there is some suggestion that TD 17β-estradiol can result in a better linear growth profile, although this has not been studied in any detail to date (79).

Moreover, in the aim of being as physiological as possible, 17β-estradiol replacement therapy gives the advantage of being titrated according to estradiol serum levels that can be easily measured during treatment (58). In addition, studies conducted in young women with POI receiving 17β-estradiol regimens showed a reduction in blood pressure, significantly greater changes in spinal bone mineral density (BMD) and improvements in uterine parameters than EE treated peers (80; 81; 82; 83). Moreover, increased C-reactive protein and acute phase reactants, independent factors risk for cardiovascular disease, may be found during EE regimens administration (84).

The administration of 17β-estradiol can be either oral or transdermal (TD). 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 estrogens, usually observed after the oral route (85). Moreover, the matrix composition of the new generation TD patches allows the cutting into pieces in order to start induction at very low doses. In addition, with a patch, it is also possible to monitor the duration of attachment (that is the duration of HRT) thus mimicking the normal diurnal/nocturnal variations in 17β-estradiol (58; 86). All in all, studies conducted in TS patients concluded that the total estrogen exposure can be significantly higher after oral 17β-estradiol, thus TD 17β-estradiol assures a more physiological estrogen milieu (87). In other studies, TD 17β-estradiol was associated with more effective feminization than oral estradiol (88) with a greater increase in uterine dimensions and greater breast development (89; 90). Indeed, the first randomized control trial comparing oral versus TD estrogen performed by Nabhan et al showed that TD estrogen use resulted in faster bone accrual and increased uterine length and volume, as well as a trend towards breast development. On the other hand, there were no significant differences in IGF-I levels, lipid profiles, growth velocity, BMI and fat mass between the two treatment groups (89). The second randomized

control trial was performed by Jospe and coll to compare TD and oral estrogen therapy in TS patients in terms of liver metabolism. They concluded that, especially in girls with TS, estrogen replacement by the TD route may have less deleterious effect on hepatic metabolism than oral estrogen (91). In addition, some studies have raised the hypothesis that the TD route could be not only more physiological, but safer from the cardiovascular and metabolic point of view. This is far from being clear; nonetheless, the oral route seems to be associated with a procoagulant state (92). On the other hand, oral 17β-estradiol can show more beneficial effects on lipids (reducing LDL and raising HDL) than transdermal which is only associated with cholesterol reduction (93). Moreover, in 2004 Mauras and coll found that neither oral nor TD estrogen adversely affect protein turnover, lipolysis, plasma lipids, fibrinogen, fasting insulin concentrations or IGF-I concentration (94).

1.2.1.3 17β-estradiol induction regimens

Regarding TD 17β-estradiol pubertal induction, to date several different regimens have been described. Cisternino and coll., in 1991, described one of the first studies on pubertal induction using TD 17β-estradiol patches in 15 females affected with HH or POI. Patients were initially given 25 μg transdermal 17β-estradiol patches two times a week, subsequently increased till the introduction of progesterone, after 6 months. The overall induction lasted from 0.5 to 3 years with consequent breast development, vaginal maturation, increase in uterine size and achievement of pubertal uterine shape (95). Later on, Ankarberg-Lindgren et al. developed dosing guidelines for TD 17β-estradiol based on the initial overnight application of patches to mimic the nocturnal activation of the hypothalamic-pituitary-gonadal axis and to resemble the diurnal estrogen fluctuation typical of spontaneous early puberty. Dosing regimen was based on body weight and dose adjustment was carried out by measuring serum estradiol levels using an ultra-sensitive assay, when available, or clinical assessment of breast development. Nonetheless, the dosing guideline did not extend beyond early to mid-puberty (57). On the

contrary, in the study by Nabhan et al., TD 17β-estradiol were started at higher doses with 25 μg to be increased to adult doses of 100-150 μg (89).

Davenport, on the other hand, provided a recommendation for pubertal induction in girls with Turner syndrome basing TD 17β-estradiol dose on body weight in early puberty, and relating 17β-estradiol doses to target serum estradiol levels. The regimen included typical adult TD 17β-estradiol doses together with advice for the introduction of progestins (60).

The idea of adjusting estradiol dose according to body weight found in some of these HRT regimens, at least in the early stages of pubertal induction, is justified by the finding that serum levels of 17β-estradiol vary with body size (57). However, it may be noted that Labarta et al., comparing the use of fixed versus individualized doses of oral estradiol for pubertal induction in girls with Turner syndrome, show that a fixed dose regimen is not inferior to the individualised one (96).

Regarding oral estrogen pubertal induction, Zacharin published a regimen using oral 17β-estradiol with a starting dose of 0.5 mg every second day increasing over 2 years to an adult dose of 2 mg (50). On the contrary, Delemarre et al. (97) and Bannink et al. (98) based 17β-estradiol dose on body weight, starting with 5 μg/kg/day and increasing to an adult dose over 2 and 3 years respectively. Finally, Labarta et al. used 0.2 mg 17β-estradiol daily for one year, followed by 0.5 mg daily for the second year (96).

Dose titration during induction represents a controversial issue, Ankarberg-Lindgren and coll suggest monitoring serum 17β-estradiol concentrations in early puberty with an ultrasensitive assay, aiming for serum concentrations in the early pubertal range of 10–40 pmol/L, given the considerable interindividual variation in serum 17β-estradiol concentrations even when using weight adjusted regimens (57; 58). On the other hand, in the study of Bannink and coll, serum estradiol concentrations do not provide additional information on the progression of puberty

(98). In case of completed puberty, a pharmacokinetic and pharmacodynamic study of oral and transdermal 17β-estradiol in TS girls, deriving adult 17β-estradiol concentration from healthy menstruating adult women with regular menses, suggested a target of 350 pmoL/L (93).

When considering ERT, it is important to take into account not only type, route and dosage but also tempo. Indeed, delaying pubertal induction can be detrimental to bone, uterine, and psychosocial health parameters (61). Moreover, prepubertal growth velocity is greater in the appendicular than in the axial skeleton. Therefore, patients with hypogonadism, especially with delayed diagnosis and treatment, frequently exhibit eunuchoid proportions (with relatively long limbs compared to the spine) given the prolonged prepubertal growth. At the start of puberty, truncal growth accelerates over appendicular, sometimes resolving disproportions (72).

In this context, whenever possible in the absence of an underlying diagnostic delay, the current literature available on the subject (even if mainly derived from studies on TS girls) suggests starting ERT in tandem with their peers at the age of 11-12 years (21) and no later than 13 years to optimize outcomes and to assure a better linear growth always in the respect of individual psychosocial and emotional maturity (59; 64; 99; 100). Although it is accepted that the clinician should decide whether to postpone pubertal induction in order to allow the possibility of spontaneous puberty to occur, postponing pubertal induction may have detrimental effects on adult height especially in specific categories, namely TS and CCS patients.

Turner syndrome, indeed, is usually associated with decreased growth rates during infancy and childhood as well as lack of pubertal "growth spurt", resulting in an adult height approximately 4 SD below the mean in untreated patients and 2 SD in rGH treated pears (101; 102). Although the underlying mechanism of growth failure in TS remains to be defined, it probably results from an impaired response to growth hormone combined with an underlying skeletal dysplasia. In this context, early initiation of low-dose estrogen along with rGH therapy can improve adult height (100). Moreover, CCS exposed to cranial, craniospinal or TBI present with an impaired

adult height due either to direct radiation damage on growth plates (craniospinal and TBI) or to iatrogenic growth hormone deficiency, when cranial and craniospinal irradiation include the HP region (103): postponing pubertal induction may result in an additional worsening of the stature attained at the end of growth and segmental disproportion.

On the other hand, in CHH a delayed growth spurt on the background of otherwise preserved linear growth can lead to both taller stature (104) and segmental disproportion (105).

1.2.2 Progestins

Although estrogen-only pubertal induction is possible, there is some evidence that an unopposed estrogen regimen could expose 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 (106; 107). To date, in all the combined estrogen/progesterone regimens available in literature, the latter is introduced after a preset period (usually 18-24 months of ERT) or at bleeding occurrence, without any individualization regarding breast and uterine ERT response. Although, this approach is mainly based on expert practice and does not rely on randomized studies. Moreover, there is some suggestion that an early administration of progesterone, especially the more androgenic agents, may compromise future breast and uterine growth and development (108; 109). In this context, it seems necessary to individualize progesterone introduction at bleeding occurrence, but only if adult breast and uterine conformation has been achieved. Indeed, if symptoms of endometrial hyperplasia occur when breasts or uterus are not yet fully developed, a reduction in 17β-estradiol dose should be advisable instead of introducing the progestin. Nonetheless, this approach at present relies only on expert practice and has not been proven in clinical studies yet (55).

As far as type of progesterone is concerned, to date, Medroxyprogesterone acetate (MPA) is the only progestin for which available evidences demonstrate the full effectiveness in inducing secretory endometrium when associated to a full replacement dose of estrogen (110; 111). Although it has been showed that MPA, having androgenic effects, may negatively impact on the cardiovascular risk given its role on lipid profiles, vasomotion and carbohydrate metabolism more than alternative treatment options, many authors consider MPA as the first choice in the setting of HRT, given the wide availability of data about its efficacy and the lack of data about whether these aspects eventually translate into cardiovascular events or not (112).

An increasing body of literature to date has demonstrated the advantages of using natural micronized progesterone (MP) in the setting of HRT, due to its reduced hormonal-related cardiovascular risks when compared to synthetic progestins (80) and its neutral/beneficial effect on blood pressure (113). Moreover, MP seems to be safer even in terms of thrombotic risk (114), even though this has not been studied in the POI population yet. Even recognizing that further studies on efficacy of MP are needed, the European Society for Human Reproduction and Embryology (ESHRE) included MP among the recommended progestins for HRT in adult women with POI (115).

When considering other progestins, the enhanced oral bioavailability of the synthetic dydrogesterone (116) has led it to be included among the suggested progestins by the ESHRE consensus guidelines (115) nonetheless no studies so far have analysed its endometrial effects in patients with hypogonadism. Moreover, it has to be taken into account that some synthetic progestins, differently from the natural micronized progesterone, showing an affinity for estrogen or androgen receptors, can have additional effects. Among them, for instance, the more androgenic agents, such as norethisterone, seem to interfere with uterine maturation and development (108; 117).

Progestins could be administered with a sequential or a continuous regimen, inducing or preventing withdrawal bleeding, respectively. In both cases, the dose of progestins is based on the concurrent dose of estrogen administered.

One of the advantages of the sequential administration is that it would allow an earlier recognition of a pregnancy in the absence of bleeding: indeed, as aforementioned, there are rare but possible cases of remitting HH or ovulation in POI.

In this setting, combined oral contraceptive (COC) pills could be used in patients needing contraception or rejecting HRT, for lower social acceptance. Indeed, spontaneous ovulation and conception are extremely rare but could occur in patients with POI with a reported incidence of 20–25% and 5–10%, respectively (118; 119).

As COC aims at contraception rather than HRT, estro-progestin dosages can be higher than in HRT regimens, suggesting the possibility that they could be associated with an increased thromboembolic risk (80). To date, however, no randomized trials comparing HRT and COC in terms of cardiovascular and thromboembolic risk, quality of life assessment or bone health have been designed specifically for hypogonadal girls and clinical recommendations are obtained from post-menopausal women. Moreover, COC formulations usually contain EE, with all the aforementioned related issues. Nonetheless, recently, the scientific community has shown an increasing interest in natural estrogens such as 17β-estradiol or its valerate ester, but whether COC with estradiol are safer than those formulated with EE in terms of thromboembolic risk is still to be established (120; 121).

Even though in a double-blind randomized study Gaussem and colleagues showed fewer negative influences on coagulation and fibrinolysis of 17β-estradiol/nomogestrol acetate COC than EE/levonorgestrel ones (122), the clinical impact of these findings remains to be demonstrated in terms of venous thromboembolism incidence.

European consensus guidelines for hypogonadism do not recommend the COC therapy (7) even though these continue to be often prescribed. The aforementioned emerging data show that COC therapy is less favourable in BMD improvement, uterine parameters and long-term cardio-metabolic health compared with a physiological estrogen replacement, particularly in older females. Moreover, in hypogonadal girls, packs should generally be taken back-to-back to avoid the risk of re-exposure to hypogonadism for 1-week-in-4 (76; 80; 81; 123).

1.2.3 Induction of Puberty in CCS

In girls with hypogonadism the optimal individual estrogen/progesterone treatment route, drug, dose and dosing tempo to reach an adequate growth, feminization and mineralization have still to be established. Moreover, no studies to date 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 secondary to different aetiologies rather than tailored on the specific clinical features and needs of the population of cancer survivors (Table 6, (40)).

As aforementioned, several studies demonstrated a reduced uterine development in patients treated with EE for pubertal induction (90; 124). Conversely, TD 17β-estradiol has shown better results in terms of uterine parameters (82). Given the well-recognised detrimental effects of pelvic irradiation on uterine maturation (63), it should be advisable to choose the most effective ERT for pubertal induction in CCS. Moreover, since infertility represents a frequent consequence of cancer treatments, the advancements in assisted reproductive technologies (including fertility preservation and oocyte donation) lead to a new need to focus on uterine maturation due to the increased number of miscarriages encountered in irradiated patients with suboptimal induction outcomes (65).

In addition, CCS are at increased risk of low BMD due to cancer-related treatments (namely corticosteroids or tyrosine-kinase inhibitors or TBI). Considering the aforementioned importance of adolescence in the achievement of peak bone mass, the relative contribution of each agent of HRT in pubertal induction should be taken into consideration. In this context, as previously mentioned, 17β-estradiol seems to be significantly more effective than EE (81).

Moreover, patients treated with HSCT are at higher risk of developing metabolic syndrome (125). Even in this setting, 17β-estradiol showed a better safety profile with a demonstrated reduction of LDL and concomitant increase in HDL levels (93), with EE has been shown to negatively affect mean blood pressure and insulin tolerance (126).

Furthermore, in CCS it is extremely important to choose a therapeutic route that avoids the "hepatic first-pass effect" as liver focal nodular hyperplasia (FNH) has an estimated incidence significantly higher than the general population. Given the potential contribution of ERT on the onset of this condition (127), the use of TD 17β-estradiol may be advisable, even though no specific trials have been carried out so far. Nonetheless, even patients with iatrogenic hepatic toxicity may benefit from the avoidance of liver metabolism.

Moreover, CCS frequently experience GH deficiency as a late effect of treatments undertaken and IGF-I is a useful guide in the process of titration and monitoring of rGH treatment. The use of TD 17β-estradiol, not influencing IGF-I levels (77), appears more suitable for girls with concomitant GH deficiency and hypogonadism.

Considering all the aforementioned aspects, TD 17β-estradiol appears to be theoretically the first choice to induce puberty in the specific setting of cancer survivor girls, while oral 17β-estradiol should be restricted to conditions in which the TD route is contraindicated (such as poor compliance, local reaction or chronic skin GvHD that may affect absorption).

Nonetheless, clinical trials in the specific field of HH in CCS are badly needed to confirm these assumptions.

1.3 Clinical Registries

Clinical registries are designed to systematically collect clinically relevant data about specific diseases or health issues according to standard procedures and definitions. Given the underlying epidemiological construct, they were first designed to measure health outcomes in different centres for epidemiological and health planning purposes, nonetheless, providing information of patient care of different diseases, they can be crucial to improve clinical practice. Indeed, randomized controlled trials, being conducted under idealized and rigorously controlled conditions, represent undoubtedly the gold standard of medical research but sometimes they cannot be confirmed in the real world (128). On the other hand, registry can provide valid long-term comparative effectiveness data in clinical practice. This is even more important in the context of pubertal induction in which randomized trials are no longer allowed in the absence of any estrogen preparation specifically licensed for the paediatric population (129). Nowadays, several clinical registries are available in order to collect data about different diseases which could be subsequently extracted and used to develop clinical studies. To date several different registers have been created. In the specific context of hypogonadism, for instance, some countries have established a national register of Turner syndrome. To the best of our knowledge, clinical registers including specifically hypogonadal CSS have yet to be established.

2. AIM OF THE STUDY

The optimal estrogen/progesterone induction regimen, capable of being both physiological and effective, is still to be established.

At this aim, we created a multicentre clinical registry to retrospectively analyse longitudinal data on pubertal induction of girls with congenital or acquired HH or POI in order to insight into auxological and uterine outcomes in the light different underlying diagnosis and regimen used.

3. SUBJECTS 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 Europe. At the present, five centres have accepted to participate:

- Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Università degli Studi di Milano, Milano, Italy;
- Istituto Auxologico Italiano, Università degli Studi di Milano, Milano, Italy;
- Fondazione MBBM, Clinica Pediatrica, Ospedale San Gerardo di Monza, Università degli Studi di Milano Bicocca, Monza, Italy;
- Ospedale Pediatrico Bambin Gesù di Roma, Università degli Studi di Roma "Tor Vergata", Roma, Italy;
- The Royal Marsden Hospital, Sutton, Surrey, UK.

Patients

In the last year, 106 girls with hypogonadotropic hypogonadism or premature ovarian insufficiency (chronological age, CA >10.9 years) have been added to the register.

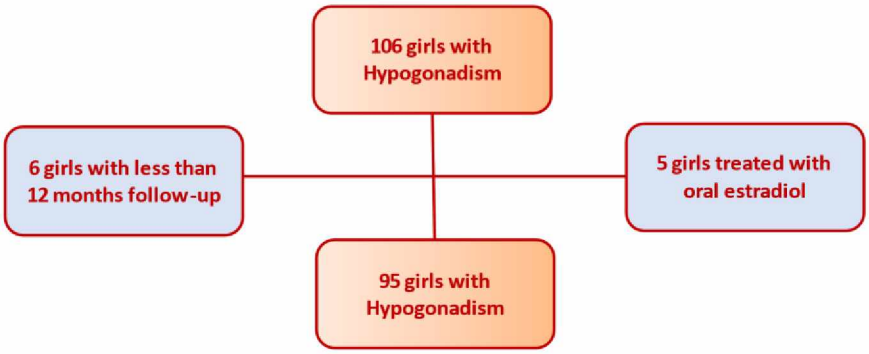
The register included girls affected with POI or HH of all causes (either genetically inherited or acquired, isolated or associated with other multiple pituitary hormone deficiencies), aged >10.9 years and treated with EP therapy for pubertal induction from January 2000. Parental written informed consent was obtained for all study patients.

All girls with uterus aplasia or arrested puberty (Tanner stage >2) were excluded.

Out of 106 patients included, we considered only 95 girls (median age 13.5 years, range IQ 12.4-15.5 years) treated with TD 17β-estradiol patches (Estraderm MX, Demestril or Evorel) with a follow-up of at least one year (median 3.3 years, range IQ 2.0-4.9 years).

All patients with shorter follow-up and treated with oral estradiol were excluded (Fig.3).

Fig.3 Exclusion process



Methods

For all study patients auxological (height, growth velocity, weight, BMI and Tanner stage), biochemical (serum estradiol levels) and radiological (hand and wrist X-ray and pelvic ultrasound) data were collected at baseline and during follow-up. 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 (130).

In all centres, pubertal development was assessed by Paediatric Endocrinologists and compared to Tanner stages (131).

Hand and wrist X-ray scan for bone age was assessed according to the standard of Tanner-Whitehouse (132) or Greulich & Pyle methodology (133). Data about uterine longitudinal diameters were obtained from pelvic ultrasounds.

Mid-Parental Height (MPH) was calculated as [(mother height + father height) -12]/2 for all study patients. Height loss was defined as the difference between the recorded height SDS at specific time points (at baseline and at adult height, AH) and MPH SDS.

Adult height was considered in the presence of a height velocity < 1 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 (134). Nonetheless, especially in patients who have experienced abdominal RT, it would be important to appreciate uterine shape in its entirety rather than relying on a single measurement. In literature, there is no consensus yet about criteria comprising global uterine maturity, though the change from tubular (pre-pubertal) in "pear-shaped" with the parallel increase of the corpus/cervix ratio is usually considered a marker of appropriate ERT response (62).

All patients underwent TD 17β-estradiol therapy (Estraderm MX, Demestril or Evorel) at incremental dosage every six months according to the clinical practice of the specific Centre. Patches were divided and conserved as previously described (135).

In 61/95 patients, progesterone (24 Dienogest, 27 Medroxyprogesterone acetate, 4 Micronized progesterone, 2 Norelgestramina, 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, after a median of 2.2 years of induction (range IQ 1.56-2.87 years).

Induction was considered completed in 49/95 patients who have:

- introduced combined oral contraceptive (COC) therapy;
- introduced progesterone during TD 17β-estradiol 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

Statistical analysis was performed using SAS (versione 9.4, SAS Institute, Cary, NC, USA). Descriptive analysis was used to characterise the study population: mean and standard deviation for normally distributed continuous variables (Shapiro-Wilks normality test), median and range interquartile (IQ) for others.

To compare normally distributed continuous variables, Student's t or ANOVA tests were used; otherwise, Mann-Whitney or Wilcoxon tests were employed.

Categorical variables were expressed as absolute frequency and percentage and compared with the $\chi 2$ or Fisher's exact test.

Auxological outcomes (adult height and mean tempo at B3 and B4) were evaluated in all study cohort. Otherwise, all uterine endpoints (uterine longitudinal diameter, uterine maturation, uterine length over 65 mm), B4 and B5 achievement were assessed in the only patients with completed induction.

In order to evaluate the association between variables of interest and dichotomous outcomes (uterine length over 65 mm, uterine maturity in shape, B4 and B5 achievement) log-binomial regression was performed (or Poisson regression with a robust error variance) to estimate the prevalence ratio (PR) and its confidence interval at 95% (CI 95%). For continuous outcomes (uterine longitudinal diameter, adult height, mean tempo at B3 and B4) was implemented a linear regression model.

Univariate linear regression analysis was used to insight the association between auxological and uterine outcomes and potentially related variables.

A multiple regression model with stepwise selection of variables was used to assess predictive and note clinical confounding factors of uterine longitudinal diameter at the end of induction. Stepwise removal was performed with exclusion criterion $P<0.10$. For longitudinal diameter at the end of the induction multiple regression model was implemented after correction for pelvic RT.

Statistical significance was defined as a two-sided $P<0.05$.

4. RESULTS

4.1 *Baseline characteristics*

All the 95 patients included in the study were affected by hypogonadism (35 with hypogonadotropic hypogonadism and 60 with premature ovarian insufficiency). In 45/95 patients (47%) HPG impairment was a late effect of cancer treatments, including surgery and/or radiotherapy of HP region (CancerHH, n=7), total body irradiation (TBI, n=36), craniospinal irradiation (n=2) and gonadotoxic chemotherapy (CancerPOI, n=38, Table 7).

Table 7. Childhood Cancer Survivors (n=45)

Childhood Cancer Survivors		N=45	
CancerHH	**n=7**	**CancerPOI**	**n=38**
Craniopharyngioma	5	Bone Marrow Transplant	35
Dysgerminoma	1	Ependymoma	1
Germinoma	1	Medulloblastoma	1
		Neuroblastoma	1

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 MPHD (MPHD, Table 8).

Table 8. Patients with central hypogonadism, congenital isolated (CHH) or associated with other multiple pituitary hormone deficiencies (MPHD).

CHH	n=22	Gene	n	MPHD	N=6	Gene2
Charge Syndrome	2	CHD7	2	**Pituitary stalk interruption syndrome/pituitary hypoplasia/ectopic neurohypophysis**	6	Unknown
Kallmann Syndrome	9	Unknown	3			
		FGFR1	4			
		ANOS1	1			
		GnRHR	1			
Normosmic HH	11	Unknown	5			
		IL17RD	1			
		WT	2			
		TACR3 omozygous	1			
		SOX10	1			
		WDR11	1			

Moreover, in 15/45 patients (16%) ovarian failure was due to X chromosome abnormalities (confirmed at Karyotype analysis) in the spectrum of Turner syndrome (TS), whereas in 7/95 (7.5%) POI was secondary to monogenic, autoimmune or unknown origin (Table 9).

Table 9. Premature ovarian insufficiency

POI	n=7	TS	n=15
Unknown	6	45, X0	3
FSHR inactivating mutation	1	46,X,i(Xq)	5
		Mosaicism SRY(+) with gonadectomy	3
		45X0/46Xr(X)	3
		45X0/46Xi(Xq)	1

Baseline characteristics of the overall study population are listed in Table 10.

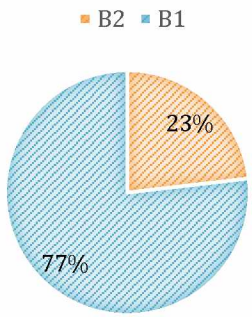
Table 10 Baseline characteristics (n=95)

Data	Mean/Median	SD	range IQ
CA[1] (years)	13.5		[12.4-15.5]
MPH[2] (cm)	164.4	4.8	
MPH[2] (SDS)	0.18	0.81	
HT[3] (cm)	149.1	11.8	
HT[3] (SDS)	-1.21	1.37	
weight (kg)	44.2		[36.1-57.4]
BMI[4] (kg/m2)	20.6	4.22	
BMI[4] (SDS)	0.14	1.47	
Baseline estradiol (ng/L)	5		[5-17]
Longitudinal uterine diameter baseline (mm)	35		[26.5-40]
BA[5] (years)	11.68	1.92	
BA[5]-CA[1]	-1.73		[-2.96 - -0.78]
BA[5]/CA[1]	0.86	0.1	
Δ HT[2]-MPH[3] bas (cm)	-14.51	11.37	
Δ HT[2]-MPH[3] bas (SDS)	-1.37	-1.37	

Chronological age[1]; Height[2]; Mid-parental height[3]; Body mass index[4]; Bone age[5]

All study patients at baseline were Tanner stage ≤ 2 as indicated in Fig.4.

Fig.4 Tanner stage at diagnosis



When considering the different underlying diagnosis, it is worth mentioning that baseline 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 reduced 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.

Out of 95, 27 girls (28%) were 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 baseline characteristics are reported in Table 11.

Table 11. Baseline characteristics according to underlying diagnosis.

Data	Cancer HH (n=7)	Cancer POI (n=38)	CHH (n=22)	POI (n=7)	TS (n=15)	MPHD (n=6)	P-value
CA[1] (years)	12.5 [12-12.6]	13.0 [12.3-14.0]	16.7 [16.0-17.9]	15.6 [15.2-16.4]	12.5 [11.9-13.8]	12.4 [11.7-13.2]	<.0001
MPH[2] (cm)	159.9(3.3)	163.8(4.4)	166.6(4.8)	164.8(3.7)	163.8(5.4)	164.7(5.2)	0.063
MPH[2] (SDS)	-0.55(0.55)	0.08(0.74)	0.55(0.82)	0.26(0.6)	0.09(0.91)	0.24(0.88)	0.073
HT[3] baseline	141.0(8.8)	145.8(7.8)	161.5(8.1)	161.7(7.4)	136.9(5.5)	150.1(10.4)	<.0001
HT[3] (SDS)	-1.86(1.26)	-1.5(0.89)	-0.15(1.2)	0.08(0.96)	-2.66(0.73)	-0.42(1.79)	<.0001
weight (kg)	48.4 [30-57.4]	40.4 [33-49.3]	56.9 [47-62.4]	49 [44.5-59.6]	36.8 [33-43.5]	47.4 [36.5-58]	0.001
BMI[4] (kg/m2)	22.9 [18.7-26.5]	18.7 [16.2-25]	20.0 [18.5-23.1]	18.7 [17.4-23.2]	19.1 [17.9-23]	20.1 [18.4-25.3]	0.573
BMI[4] (SDS)	1.51(1.75)	0.06(1.52)	-0.16(1.29)	-0.08(1.54)	0.15(1.1)	0.45(1.92)	0.183
Baseline serum estradiol (ng/L)	15.1 [5-17.3]	5 [5-18.4]	5 [5-11.4]	5 [5-19]	5 [5-15.9]	9.3 [5-23.4]	0.793
Longitudinal uterine diameter baseline (mm)	35 [25-41.5]	37 [31-43]	34 [23-40]	29 [27-45]	34.25 [27-35]	39 [27-40]	0.627
BA[5] (years)	10 [7.9-10]	11.5 [10.5-12]	13 [12.1-14.5]	12.75 [12-13.85]	11 [10.25-12.75]	9.7 [8-10]	<.0001
BA[5] -CA[1] (years)	-2.6 [-3.8--1.8]	-1.38 [-1.9--0.7]	-3.1 [-4.0--2.0]	-2.8 [-3.9--1.7]	-0.8 [-1.2--0.5]	-2.9 [-3.0--2.7]	0.001

Chronological age[1]; Mid-parental height[2]; Height[3]; Body mass index[4]; Bone age[5]

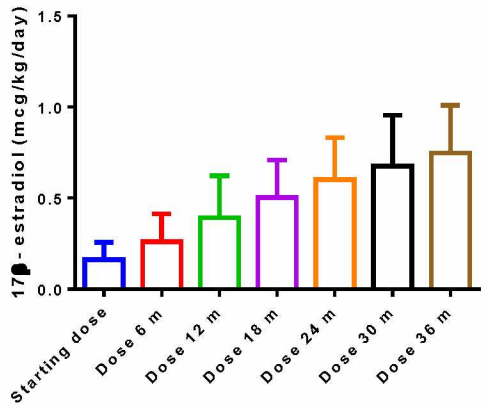
4.2 *Induction regimen*

All study patients started with low-dose TD 17β-estradiol (median 0.14 mcg/kg/day, range IQ 0.1 to 0.2 mcg/kg/day) to be increased every six months (Table 12, Fig.5). In 16/95 (17%) induction was started with overnight-only TD 17β-estradiol (with patches applied from 10:00 pm to 8:00 am). All patients were followed for at least 1 year of ERT (median 3.3 years, range IQ 2 to 4.9 years).

Table 12. Induction regimen

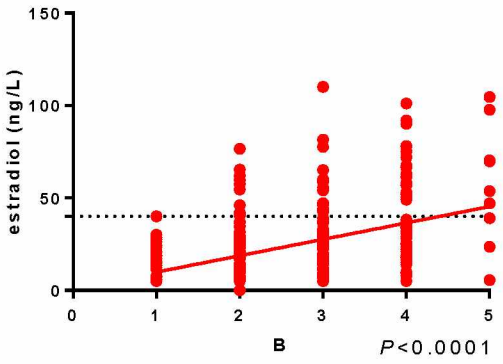
	Dose 17β-estradiol/24 h, median [range IQ]	Dose 17β-estradiol/kg/24 h, median [range IQ]
Start	6.25 [5-8.33]	0.14 [0.1-0.2]
6 months	12.5 [6.25-12.5]	0.24 [0.14-0.32]
12 months	12.5 [12.5-25]	0.34 [0.22-0.5]
18 months	25 [18.75-25]	0.49 [0.35-0.63]
24 months	25 [25-37.5]	0.57 [0.44-0.74]
30 months	31.25 [25-50]	0.63 [0.5-0.84]
36 months	37.5 [25-50]	0.75 (0.26)

Fig.5 Induction regimen



As expected, TD 17β-estradiol dose was positively correlated with Tanner stages (P<0.0001) with Tanner stage 1 for estradiol serum levels under 40 ng/L (Fig.6).

Fig.6 Estradiol serum levels and Tanner stages



Moreover, serum estradiol levels followed TD 17β-estradiol increase (Fig.7) with a direct association between the two variables at any time of the induction (P<0.0001, Fig. 8).

Fig.7. Serum estradiol levels at TD 17β-estradiol increase

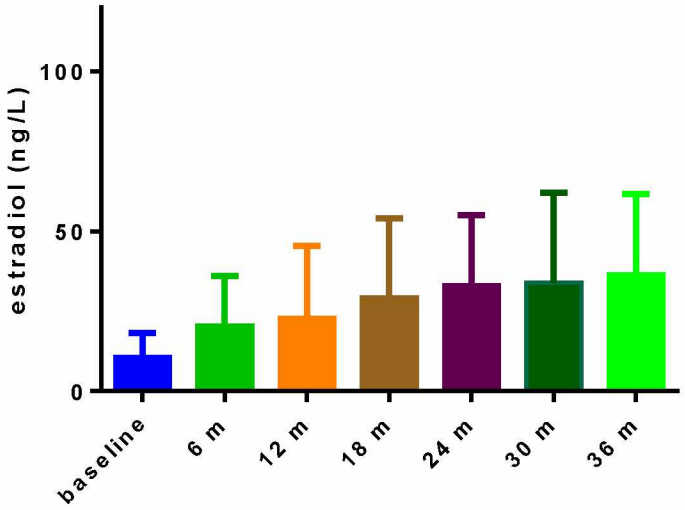
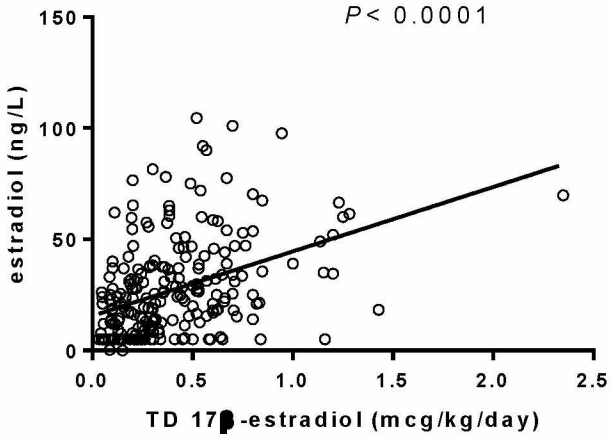
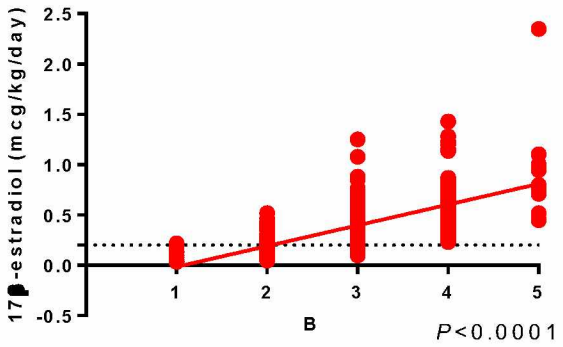


Fig.8 Serum estradiol levels at TD 17β-estradiol increase (linear regression)



As expected, TD 17β-estradiol dosage at any time of induction was directly associated with Tanner stages (P<0.0001), with Tanner stage 1 for 17β-estradiol dosage under 0.2 mg/kg/day (Fig. 9).

Fig.9 17β-estradiol and Tanner stages



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 tempo at bleeding occurrence was 2.3±1 years.

4.3 *Auxological outcomes*

In all study patients, auxological outcomes are listed in Table 13.

Table 13. Auxological outcomes (n=95).

Data (n=95)	Mean/Median	SD	range IQ
Δ B3 (years)	1.2	0.6	
Δ B4 (years)	2.1		[1.6-2.7]
Δ B5 (years)	3.9	1.8	
AH (cm)	159.8	10.2	
AH (SDS)	-0.6	1.7	
Δ AH-MPH (cm)	-4.1	9.6	
ΔAH-MPH (SDS)	-0.7	1.6	

B: Breast stage; AH: Adult height; MPH: Mid-parental height

When considering the underlying diagnosis, no difference was found in mean tempo at B3, B4 and B5. Nonetheless, patients with TS showed a reduced AH (P<0.0001), with a higher MPH distance (P=0.001).

All data are reported in Table 14.

Table 14. Auxological outcome according the underlying diagnosis

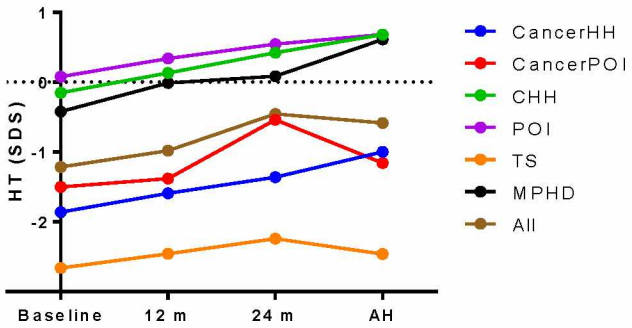
Data	Total (n=95)	CHH (n=22)	POI (n=7)	TS (n=15)	Cancer HH (n=7)	Cancer POI (n=38)	MPHD (n=6)	P-value
ΔB3 (years)	1.2 [0.79-1.64]	1.36 [0.87-1.66]	0.92 [0.66-1.33]	1 [0.66-1.48]	1.42 [0.46-1.65]	1.07 [0.73-1.72]	1.79 [1.01-2.11]	0.462
ΔB4 (years)	2.05 [1.61-2.63]	2.01 [1.52-2.34]	1.81 [1.64-1.94]	1.66 [1.46-2.44]	2.61 [2.4-3.31]	2.12 [1.71-2.97]	2.8 [2.53-2.99]	0.034
ΔB5 (years)	3.13 [2.55-4.1]	2.95 [2.51-3.3]	2.65 [2.51-2.79]	4.44 [3.39-6.25]	5.31 [3.43-7.19]	4.01 [2.43-4.5]		0.108
AH (cm)	159.7 (10.25)	167.48(7.55)	167.45(7.12)	148.08(6.47)	157.18(6.58)	156.2(8.24)	167(10.64)	<.0001
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
AH-MPH (cm)	-4.14 (9.59)	0.74(8.09)	2.74(7.65)	-13.8(3.9)	-5.53(5.15)	-9.36(8.94)	4.17(7.64)	0.001
AH-MPH (SDS)	-0.68 (1.56)	0.1(1.32)	0.44(1.26)	-2.22(0.68)	-0.92(0.88)	-1.53(1.46)	0.69(1.22)	0.001

B: Breast stage; AH: Adult height; MPH: Mid-parental height

Mean tempo at B3 was influenced by delayed bone age (BA/CA ratio R^2=0.08, P=0.02) and reduced 17β-estradiol dosage at start (R^2=0.13, P=0.001) even when expresses for weight (R^2=0.08, P=0.008). Moreover, mean tempo at B4 was influenced by delayed bone age (R^2=0.12, P=0.01).

As far as linear growth is concerned, the trend of height during ERT according to the underlying diagnosis is reported in Fig.10.

Fig.10 Height gain during induction according to initial diagnosis

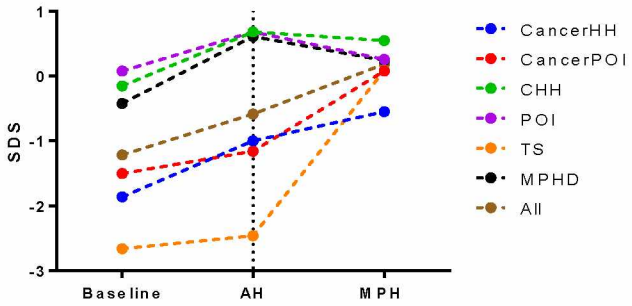


Moreover, at linear regression growth velocity during the first year of ERT resulted negatively associated with baseline chronological age (R^2=0.35, P<0.0001) and Tanner stage at baseline (R^2=0.17, P<0.0001). As far as adult height is concerned, at multiple regression a higher AH SDS was associated with lower 17β-estradiol dosage at induction

start (P=0.034, β=-4.21) and growth velocity in the first year of treatment after correction with possible confounders (bone age and chronological age at ERT start, rGH concomitant treatment and underlying diagnosis, P=0.028, β=0.14).

Moreover, ERT reduced MPH distance, being significantly different at AH from baseline (MPH distance at AH -0.68±1.56 SDS vs -1.37±1.37 SDS at baseline, P<0.0001). Nonetheless, CancerPOI and TS does not achieve their height potential, with AH SDS significantly different from MPH SDS (P=0.001 and P=0.03, respectively) despite rGH concomitant treatment in 2/38 and 14/15 cases, respectively (Fig.11).

Fig. 11 Height gain at the end of induction and mid-parental height distance according to the initial diagnosis



4.3 *Outcomes at the end of the induction*

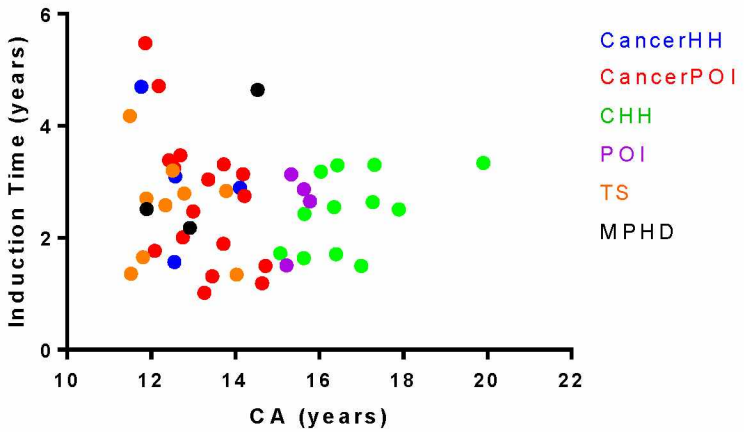
When considering patients with completed pubertal induction, outcome characteristics are listed in Table 15.

Table 15. Outcome characteristics (n=49)

Data	Total (n=49)	CHH (n=12)	POI (n=4)	TS (n=9)	CancerHH (n=4)	CancerPOI (n=17)	MPHD (n=3)	P-value
Δ Induction (years)	2.65 [1.72-3.2]	2.53 [1.72-3.24]	2.76 [2.08-3]	2.7 [1.65-2.84]	3 [2.23-3.9]	2.75 [1.77-3.31]	2.51 [2.18-4.64]	0.967
B4 achievement (%)								
no	5 (10%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	4 (24%)	0 (0%)	0.223
yes	44 (90%)	12 (100%)	3 (75%)	9 (100%)	4 (100%)	13 (76%)	3 (100%)	
B5 achievement (%)								
no	29 (59%)	3 (25%)	2 (50%)	5 (56%)	2 (50%)	14 (82%)	3 (100%)	0.023
yes	20 (41%)	9 (75%)	2 (50%)	4 (44%)	2 (50%)	3 (18%)	0 (0%)	
Uterine length > 65 mm(%)								
no	28 (62%)	7 (58%)	2 (67%)	4 (57%)	1 (25%)	13 (81%)	1 (33%)	0.264
yes	17 (38%)	5 (42%)	1 (33%)	3 (43%)	3 (75%)	3 (19%)	2 (67%)	
Uterine maturity (%)								
no	11 (26%)	0 (0%)	0 (0%)	1 (17%)	0 (0%)	10 (67%)	0 (0%)	0.001
yes	31 (74%)	11 (100%)	3 (100%)	5 (83%)	4 (100%)	5 (33%)	3 (100%)	
Final uterine longitudinal diameter (mm)	61.22 (11.1)	63(6.34)	58.33(8.62)	65(6.07)	70.5(17.94)	54.47(11.06)	70.83(11.56)	0.026
17β-estradiol dosage at progesterone introduction (mcg)	37.5 [25-50]	50 [31.25-50]	50 [50-75]	25 [25-25]	25 [25-37.5]	31.25 [25-43.75]	37.5 [25-50]	0.067
17β-estradiol dosage at progesterone introduction (mcg/kg)	0.7 [0.5-0.86]	0.79 [0.5-0.8]	1.1 [1-1.13]	0.61 [0.58-0.8]	0.58 [0.51-0.93]	0.67 [0.5-0.92]	0.51 [0.34-1.25]	0.319
17β-estradiol dosage at bleeding (mcg/die)	37.5 [25-50]	50 [31.25-50]	50 [50-75]	25 [25-25]	25 [25-37.5]	25 [25-50]	37.5 [25-50]	0.075
17β-estradiol dosage at bleeding (mcg/kg)	0.7 [0.5-0.85]	0.79 [0.5-0.8]	1.1 [1-1.13]	0.61 [0.58-0.8]	0.58 [0.51-0.93]	0.67 [0.5-0.84]	0.51 [0.34-1.25]	0.419

Induction duration was not influenced by age at start and appears to be not significantly different in the six groups (Fig.12)

Fig.12 Induction time according to chronological age (CA) and initial diagnosis



When considering feminization, 44/49 (90%) patients reached Tanner stage B4 (Fig.13) but only 20/49 (41%) Tanner stage B5 (Fig.14). Moreover, B5 achievement was significantly

associated with TD 17β-estradiol dose at progesterone introduction and number of dose changes (P=0.034) even after correction for possible confounders (Tanner stage, chronological age, BMI SDS at start and initial diagnosis).

Fig.13 Tanner stage 4 achievement

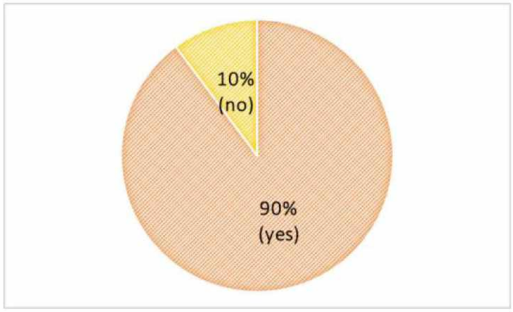
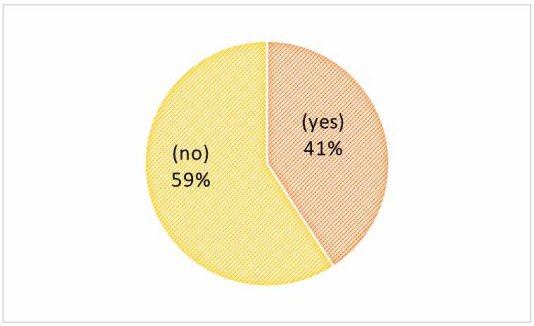


Fig.14 Tanner stage B5 achievement



Uterine longitudinal diameter showed a gradual increase during ERT (Fig.15) with a significant association with estradiol serum levels (R^2=0.2658, P<0.0001) and TD 17β-estradiol dosage (R^2=0.1249, P<0.0001) at any point of induction (Fig.16a, b). No association was found between uterine longitudinal diameter and age at start.

Fig.15

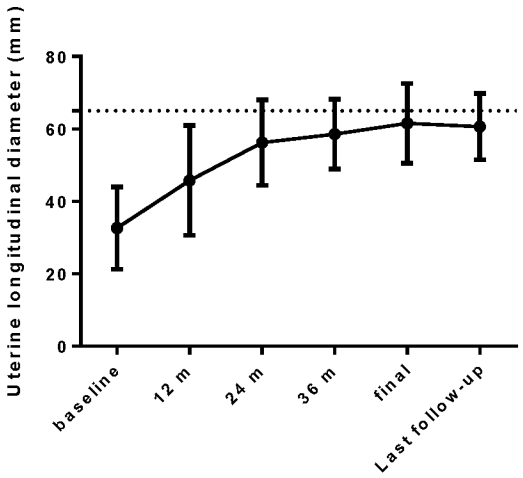


Fig.16a

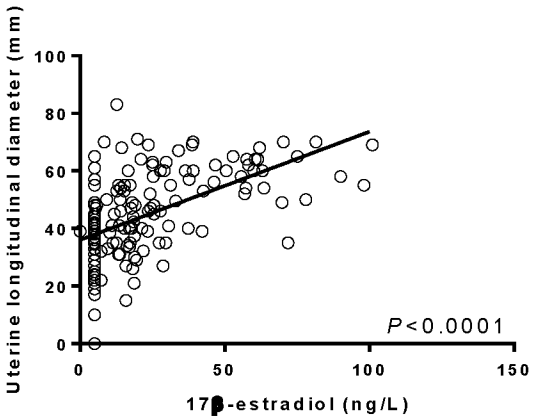
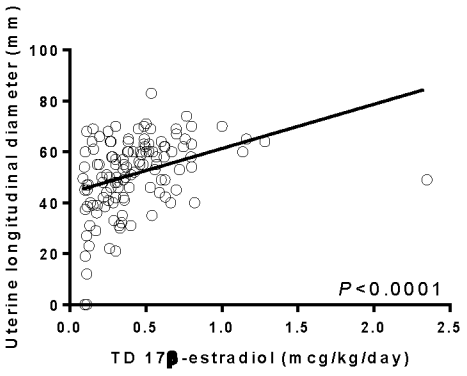


Fig.16b



At the end of the induction, 31/42 (74%) patients show a uterus with adult shape with a significant difference between patients with hypogonadism of genetic/idiopathic origin vs iatrogenic cause: indeed, other 26% was constituted of 10/11 cancer survivors and 1/11 TS (P=0.001). Despite the maturity in shape, at the end of the induction, uterus was often reduced in length (Fig.17). Indeed, only in 17/45 (38%) cases longitudinal uterine diameter was longer than 65 mm, with no significant difference between the six groups (Fig.18).

Fig.17 Uterine development at the end of the induction

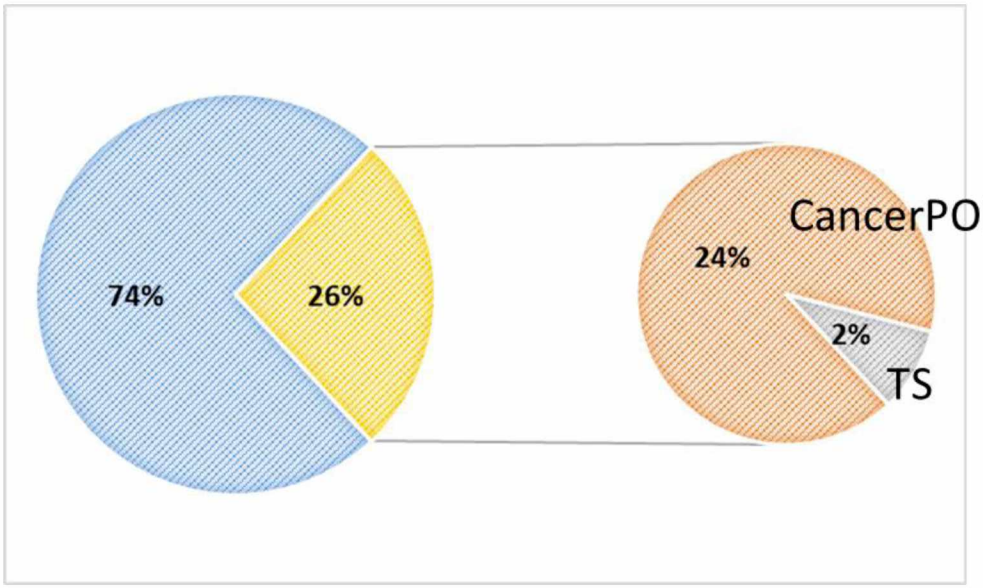
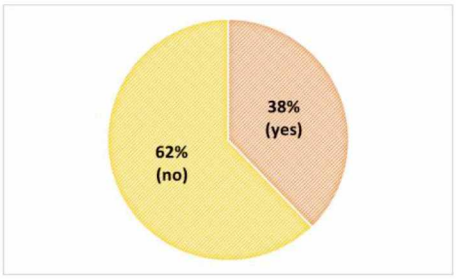


Fig.18 Uterine longitudinal diameter > 65 mm at the end of the induction

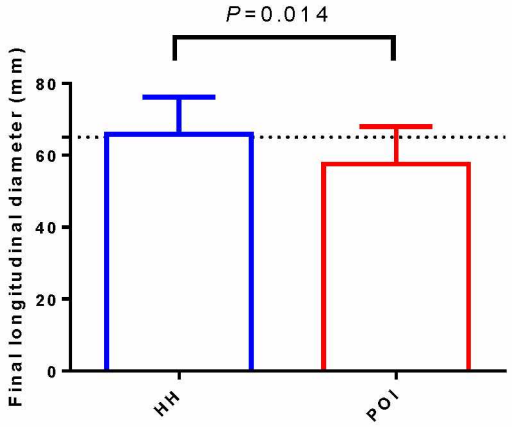


Nonetheless, uterine maturation was directly associated with uterine longitudinal diameter length at the end of the induction (R^2=0.3493, P<0.0001). It is worth mentioning that 10/15

(67%) patients with POI secondary to late effects of cancer treatment showed a uterus with tubular (immature) shape at the end of the induction (Table 15).

Final uterine longitudinal diameter was significantly higher in patients with HH (CHH, CancerHH and MPHD) rather than with CancerPOI and POI (final longitudinal diameter of 65.3±10.4 cm vs 57.6±62.0 cm, *P*=0.014, Fig. 19).

Fig.19 Final uterine diameter according to the type of hypogonadism



Indeed, at stepwise regression analysis having received TBI or pelvic RT was the major determinant of reduced uterine longitudinal diameter at the end of induction (*P*=0.034, β=-0.38). After correction for uterine irradiation, uterine longitudinal diameter was associated with 17β-estradiol dose at progesterone introduction (*P*=0.043, β=0.23), with a possibility of exceeding 65 mm in length 4% higher every unitary increase of 17β-estradiol dosage (*P*=0.045) and of 62% higher at every single additional increment in ERT dosage (*P*=0.022). Data about regression analysis are listed in Table 16.

Table 16. Regression analysis

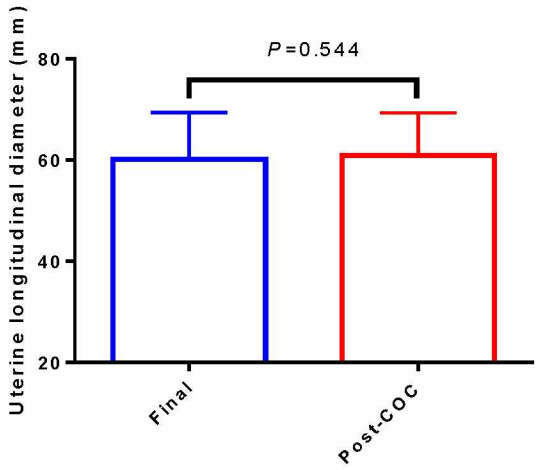
	Linear regression uterine longitudinal diameter				Log-poisson regression for uterine length >65 mm			
Variables	p-value (Univariate)	p-value (Correct*)	β	CI	p-value (Univariate)	p-value (Correct*)	(exp)β	CI
Total estrogen exposure (mg)	0.585	0.521	0.101	(-0.219;0.422)	0.529	0.045	1.045	(1.001;1.090)
Mean tempo at Pg[1] introduction (years)	0.960	0.571	0.896	(-2.320;4.111)	0.266	0.093	1.356	(0.950;1.934)
Induction time (years)	0.813	0.579	0.895	(-2.390;4.180)	0.287	0.088	1.415	(0.950; 2.106)
Dose 17βE_2[2]/24h intro Pg[1] (μg)	0.738	0.043	0.232	(0.007;0.457)	0.932	0.128	1.020	(0.994;1.046)
Dose 17βE_2[2]/24h start (μg)	0.471	0.271	-0.345	(-0.978;0.287)	0.068	0.120	0.845	(0.683;1.045)
Number of increments ERT[3]	0.579	0.155	1.892	(-0.765;4.548)	0.169	0.022	1.620	(1.071;2.451)

*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

No other difference was found between the six groups.

Uterine longitudinal diameter at the end of the induction was not significantly different from the one assed at last follow-up after at least 6 months from COC introduction (Fig.20).

Fig.20 Difference between uterine longitudinal diameter at the end of induction and after at least 6 months follow-up from combined oral contraceptive (COC) introduction



5. DISCUSSION

To the best of our knowledge this represents the first observational study on induction of puberty in different types of hypogonadism using TD 17β-estradiol including more than a half patients CCS. Indeed, until now, outcomes of pubertal induction have been collected from isolated experiences, small observational studies or small clinical trials mostly conducted on girls with TS, a population with clinical peculiarities in terms of treatment goals and risk factors. In accordance with that, in our population, baseline characteristics as well as outcomes at the end of the induction were extremely different according to the underlying diagnosis, thus leading to wonder if it is possible to use in

clinical practice information derived from studies on induction performed in other diagnostic categories which are not necessarily comparable (55).

First of all, at baseline, girls with isolated CHH and POI were relatively older than others, probably due to the important diagnostic and treatment delay. For the vast majority of HH and POI patients, indeed, the diagnosis is usually considered only after a long period of pubertal delay, reflecting either late clinical referral and frequent misapplication of management principles aligned with CDGP to adolescents with likely hypogonadism (55; 136).

Moreover, at baseline, TS and CCS were shorter than other study patients with a bone age not significantly delayed. As expected, this resulted in a worse AH, despite the reduction of MPH distance during treatment in all study groups. Indeed, as already reported in literature, TS and CCS did not achieve their genetically-determined height potential, despite rGH concomitant treatment (100; 103).

As far as adult height is concerned, 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. However, it would be interesting to see if this protracted linear growth increases body disproportion with eunuchoid proportion at AH, as previously described in male counterpart (105).

In literature, some authors suggest starting induction with low dose TD 17β-estradiol, about 0.1 μg/kg, to be applied only at night. Thereafter, ERT should be gradually increased before mid-pubertal levels are reached, in order to mimic the spontaneous estrogenic levels in the early pubertal range as well as the diurnal pattern of serum 17β-estradiol (57; 58; 60). The rationale of this cautious approach is promoting gradual breast maturation as well as increasing growth velocity and "pubertal growth spurt". Nonetheless, no clinical study has yet demonstrated any actual superiority of this

regimen or of the initial overnight treatment. In our cohort, low 17β-estradiol dose at induction start, even considering overnight-only initial ERT, seems to be beneficial in terms of height gain at AH, independently from initial diagnosis, concomitant rGH treatment or age at start. When considering these confounders, on the other hand, the most important predictor of AH was growth velocity in the first year of induction, thus remarking the importance of low 17β-estradiol growth-promoting doses at start.

Nonetheless, it remains to be ascertained if the overnight ERT in some specific categories with important diagnostic delay, namely CHH and POI, could even prolong pre-pubertal growth and therefore it should be reasonably skipped to avoid the establishment of eunucoid proportion.

Even though at different TD 17β-estradiol doses and estradiol serum levels correspond different Tanner stages, as previously described, in our cohort Tanner stage I was associated with 17β-estradiol under 0.2 mcg/kg/day as well as estradiol serum levels below 40 ng/L at any point of the induction. This is an important aspect to take into consideration in the attempt of reaching Tanner stage 3 after at least 12 months from induction start, maintaining TD ERT initial dosage under the 0.2 mcg/kg/day. Despite the different regimens used in literature, the dynamics of breast maturation is rather uniform, with the achievement of stage B2 during the first six months and B4 after approximately 2 years (137; 138), which is comparable to spontaneous puberty. As in physiological pubertal development, in our cohort mean tempo at Tanner stage 4 was 2.05 years, with 90% achieving that stage of breast development before the end of the induction. Nonetheless, only 40% patients reached Tanner stage 5, being B5 achievement significantly associated with gradual TD 17β-estradiol incremental dosage and ERT dose at progesterone introduction.

When considering uterine parameters, despite substantial changes in shape in the majority of cases, the final longitudinal diameter was reduced in length in more than a half of patients as previously reported (62). In the study of Burt et al, indeed, the final uterine longitudinal diameter of hypogonadal girls was significantly lower than the control group, with no significant difference according to induction characteristics and diagnostic categories, but with a positive correlation with serum estradiol levels (62). This compromised uterine growth could be due to inadequate estrogen replacement doses, as supported by the association found in our study group between serum estradiol levels or TD ERT dosage and longitudinal diameter length.

According to our data, the worst outcomes were found in patients who received uterine irradiation (CancerPOI) and TS. Indeed, the most important determinant of uterine maturation was the underlying diagnosis and cancer treatment itself, probably due to the presence of unchangeable characteristics (as uterine fibrosis in the specific case of CancerPOI) that can negatively influence treatment response rather independently from the induction regimen used. After correction for pelvic irradiation, TD 17β-estradiol dosage at progesterone introduction, number of dose changes and 17β-estradiol total exposure were significantly associated to a better uterine response, thus suggesting that higher levels of TD 17β-estradiol in the last part of the induction are necessary to optimize outcomes. Indeed, it has been hypothesized that there should be a critical window for uterine development during puberty, outside of which reduced size cannot be recovered (55; 62). The existence of a precise timing in uterine maturation might be suggested by the evidence that during pubertal development there is a significant correlation between uterine dimensions and estradiol concentration with more striking changes at Tanner stage B3 and B4 (62; 139; 140; 141).

This is an important aspect to take into consideration in the aim of optimizing progesterone start. Our data suggest that no further uterine development occurs after progesterone introduction, with longitudinal diameter remaining unchanged after COC start. In this context, it seems necessary to consider introducing progesterone at bleeding occurrence, but only if adult breast and uterine conformation has been achieved (62). Otherwise, if signs of endometrial hyperplasia occur in the presence of underdeveloped breasts or uterus, then a slight reduction in 17β-estradiol dose should be taken into consideration instead of introducing progestins, although this should be confirmed in studies on a larger scale.

Nonetheless, data on uterine maturation suggest that increasing TD 17β-estradiol dose could be completely useless in some specific patients with scarce uterine response to ERT in the presence of history of pelvic irradiation, albeit it could expose at higher risk of side effects.

The main limits of the present study are the retrospective nature, typical of data derived from clinical registry, and the sample size, due to the low prevalence of congenital or acquired hypogonadism of pre-pubertal onset.

6. CONCLUSION

In conclusion, our data confirm the importance of starting induction of puberty with growth-promoting TD 17β-estradiol dose, corresponding to about 0.1-0.2 μg TDE/kg/day, even considering initial overnight-only ERT. This is of particular interest in patients with worse adult height outcomes (namely TS and CCS patients) in order to optimize the eventual concomitant treatment with rGH, preventing premature epiphyseal fusion. Moreover, gradual ERT increase seems to be beneficial in terms of feminization. Indeed, reaching an appropriate TD 17β-estradiol dose at the end of the induction seems to play a crucial role in uterine development and Tanner stage 5

achievement. Furthermore, considering that adolescence represents a key window for uterine maturation, progesterone should be started only in the presence of a concomitant adequate ERT dose and an appropriate uterus and breast development, given the evidence that the introduction of progestins hampers the subsequent changes in uterus volume or the achievement of the last Tanner stage.

At present, all the induction regimen proposed are derived from data on physiological puberty, isolated experiences, small observational studies or small clinical trials. Indeed, 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 in the clinical registry to confirm present results on a larger scale.

7. APPENDIX A

CED can be calculated according to the following equation:

CED (mg/m2) = 1.0 x (cumulative cyclophosphamide dose [mg/m2]) + 0.244 x (cumulative ifosfamide dose [mg/m2]) + 0.857 x (cumulative procarbazine dose [mg/m2]) + 14.286 x (cumulative chlorambucil dose [mg/m2]) + 15.0 x (cumulative carmustine dose [mg/m2]) + 16.0 x (cumulative lomustine dose [mg/m2]) + 40 x (cumulative melphalan dose [mg/m2]) + 50 x (cumulative Thiotepa dose [mg/m2]) + 100 x (cumulative nitrogen mustard dose [mg/m2]) + 8.823 x (cumulative busulfan dose [mg/m2] (39)

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