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PREVALENCE OF PRECOCIOUS AND EARLY PUBERTY IN GIRLS FROM ELX. IS IT REALLY THAT HIGH?

-A CROSS-SECTIONAL BICENTRIC STUDY-

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1. ABBREVIATIONS

- AEPED Asociaciación Española de Pediatría
- BMI Body Mass Index
- BPA Bisphenol A
- CPP Central Precocious Puberty
- CVD Cardiovascular Disease
- ECDs Endocrine Disrupting Chemicals
- EP Early Puberty
- FSH Follicle Stimulating Hormone
- GnRH Gonadotrophin Releasing Hormone
- HLaFe Hospital Universitari i Politècnic La Fe València
- HPG axis Hypothalamic–Pituitary–Gonadal axis
- HUE Hospital General i Universitari Elx
- LH Luteinizing Hormone
- PP Precocious Puberty
- PPP Peripheral Precocious Puberty
- PU Pelvic Ultrasound

2. ABSTRACT

Background: Precocious and Early Puberty consist in the onset of the pubertal development in a non physiological age, which is defined in girls in 8 years and 8-to-9 years respectively. Although these limits ages have been recently discussed. It is estimated that 0,2 girls suffer precocious puberty and around 2% early puberty, with a female/male ratio of 20:1. Depending on the activation or not the hypothalamic–pituitary–gonadal axis we can find central precocious puberty or peripheral. New aetiologies are being discussed during the last decade due to the big part of idiopathic precocious and early puberty is being diagnosed. Apart of genetics, metabolic, social and environmental risk factors are on the table. On these last, some environmental oestrogenic substances, called Endocrine Disrupting Chemicals, seem to play an important role starting the pubertal process.

Objectives: The goal of this study is to calculate the prevalence of Precocious Puberty and Early Puberty of the girls from Elx and to compare it with the prevalence in València to demonstrate that in Elx is higher than in other cities. The second main objective is to associate this higher prevalence from Elx to the possible contact of the prepubertal girls from Elx with the footwear industry, which may be acting as an Endocrine Disruptor.

Methods: This study is a cross-sectional bicentric study to calculate the prevalence of Precocious and Early Puberty, perform at Hospital General Universitari d'Elx and Hospital Universitari i Politècnic La Fe de València. The sample size will be 876 girls from Elx and València schools selected by a probabilistic method sampling, using a cluster sampling procedure. A 3 years period is estimated.

Keywords: Precocious Puberty, Early Puberty, Endocrine Disrupting Chemicals, Pelvic Ultrasound, Footwear Industry.

3. INTRODUCTION

3.1 BACKGROUND

Puberty is the process of transition from childhood to adulthood and is an important developmental stage during which secondary sex characteristics appear, adolescent growth spurt occurs, reproductive capacity is achieved, and profound psychological changes take place(1). In boys, the first sign of pubertal development is an increase of the testicular volume above 3 ml, consistent with Tanner G2 stage. In girls, the earliest manifestation of puberty is the acceleration in growth velocity and thelarche, which is the first appearance of breast defined as Tanner B2 Stage(2,3) (ANNEX 1).

About the age, in 1985 Tanner and Davies talked about 12,7 years old the appearance of menarche, which is really close to the data obtained in most European countries (FIG.1). It is important to understand the fact that this happen in well-off conditions populations. In the other side, when we talked about underprivileged countries age increases to 16,1 years (2).

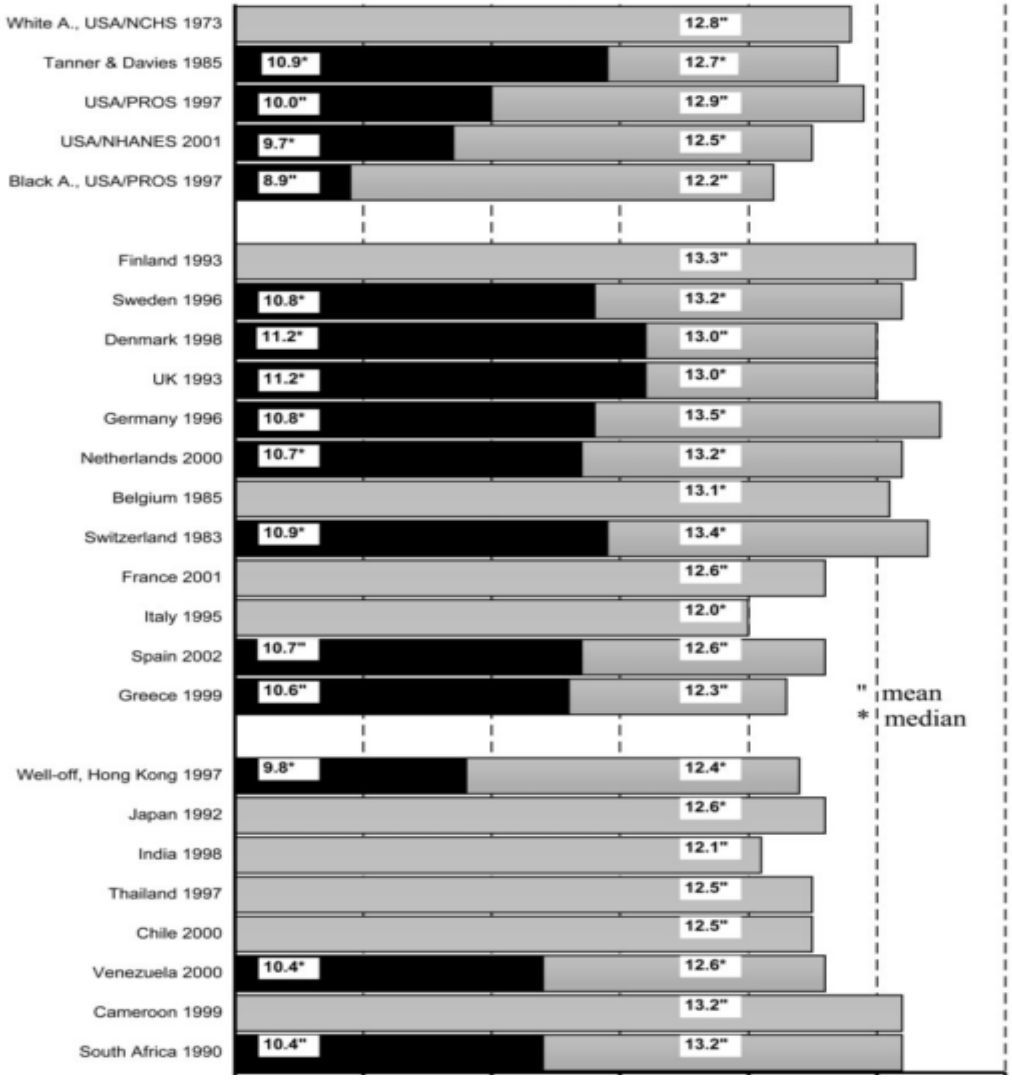

FIG.1: Average ages of thelarche (B2) or menarche in different well-off populations around the world (2).

Pathogenesis:

About the pathogenesis of puberty in general population we can define puberty as the result from the awakening of a complex neuroendocrine machinery in which the primary mechanism is still unclear(2), which involves the maturation and activation of the hypothalamic–pituitary–gonadal (HPG) axis(4).

It requires changes in the liberation of gonafotropin releasing hormone (GnRH), which is a decapeptide produced by specialized neurons that intermittently secrete pulses of hormone from nerve terminals positioned in the median eminence of the basal hypothalamus.

These changes are, in turn, determined by modifications in transsynaptic and glial inputs to the GnRH neuronal network, increasing in first place the frequency and the amplitude of the GnRH peaks secretion (around 2 years before of the first pubertal signs) during the night(5). While the transynaptic changes involve an increase in excitatory inputs and a reduction in inhibitory influences, which were the predominant until that moment, the glial component is predominantly facilitatory and exerted by both growth factors and small molecules that directly or indirectly stimulate GnRH secretion(6).

The direct excitatory transsynaptic regulation of GnRH secretion is provided by at least three different neuronal subsets: kisspetin neurons acting via GPR54 receptors, glutamatergic neurons acting mostly via AMPA receptors, but also NMDA receptors, and GABA acting via ionotropic GABAa receptors. The inhibitory counterpart of this circuitry depends principally on GABAergic neurons acting via GABAb metabotropic receptors, but also on opiatergic neurons that employ different peptides and a variety of different receptors for inhibitory neurotransmission(6).

Once liberated, GnRH enters the pituitary portal vasculature and travels to the pituitary to signal the synthesis and secretion of the pituitary gonadotropins: luteinizing hormone (LH) and follicle stimulating hormone (FSH).
Blood-borne LH and FSH act on target cells in the testes and ovaries to direct the production of sperm and eggs, as well as the secretion of steroid hormones. Gonadal steroids are vital to both gonadal functions and reproductive behaviour. Within the gonads, steroid hormones participate in spermatogenesis and follicle maturation. Within the brain, steroids influence GnRH secretion via neuroendocrine feedback loops and facilitate sexual behaviour (FIG.2).

In the pathogenesis puberty world, a perpetual goal for researchers is to find the 'trigger' that induces the re-emergence of GnRH secretion at puberty. Findings reported in scientific publications have implicated several candidates, including melatonin, body fat, leptin and most recently a single gene!

About this last or these, if we suppose that not only exists a single gene but we are talking about a set of genes, in a study of 184 pairs of monozygotic and dizygotic twin girls, breast development and age at menarche showed a high degree of genetic correlation, inferring that a common set of genes control events in puberty(7). These findings suggest that 50–80% of the variation in pubertal timing is regulated by genetic factors(8).

So, it could seems apparent that the genetic underpinnings of puberty are multigenetic, but this realization does not explain how inherited, permanent changes in DNA sequence can regulate gene expression dynamically, while also forcing an encompassing level of coordination and transcriptional plasticity to the gene networks involved, consequently it seems that timing of female puberty is under the regulatory control of an epigenetic mechanism of transcriptional repression(6).

In this epigenetical line, scientists have identified signals that permit puberty to occur or progress, but do not cause puberty. We call these ‘permissive’ signals. Not surprisingly, the permissive signals vary with species and sex, and most relate to energy balance.

The consequences of puberty, such as the defense of territory or mate, pregnancy and care of young, are energetically expensive. For this reason, the timing of puberty is critical: the individual must perceive whether it has grown sufficiently (through metabolic cues), what its relationship is to other individuals (through social cues) and whether conditions are optimal to begin the reproductive process (through environmental cues) (FIG.2).
For example, metabolic fuel availability, such as insulin, glucose and leptin in females serve as important signals to support that pregnancy can take place. Sensors in the hypothalamus and hindbrain monitor these signals and permit high-frequency GnRH release when the signals reach appropriate levels.
For many seasonal breeders, the photoperiod signals the optimal time of year for puberty onset. The circadian clock in the suprachiasmatic nucleus measures day length by controlling melatonin production in the pineal gland. The duration of the nocturnal elevation in melatonin encodes day length, and melatonin receptors in the thalamus and hypothalamus transduce this signal to GnRH neurons(9).

So we can conclude that the wide variations among the timing and tempo of normal puberty among normal individuals throughout the world are influenced by both genetic and environmental factors(1).

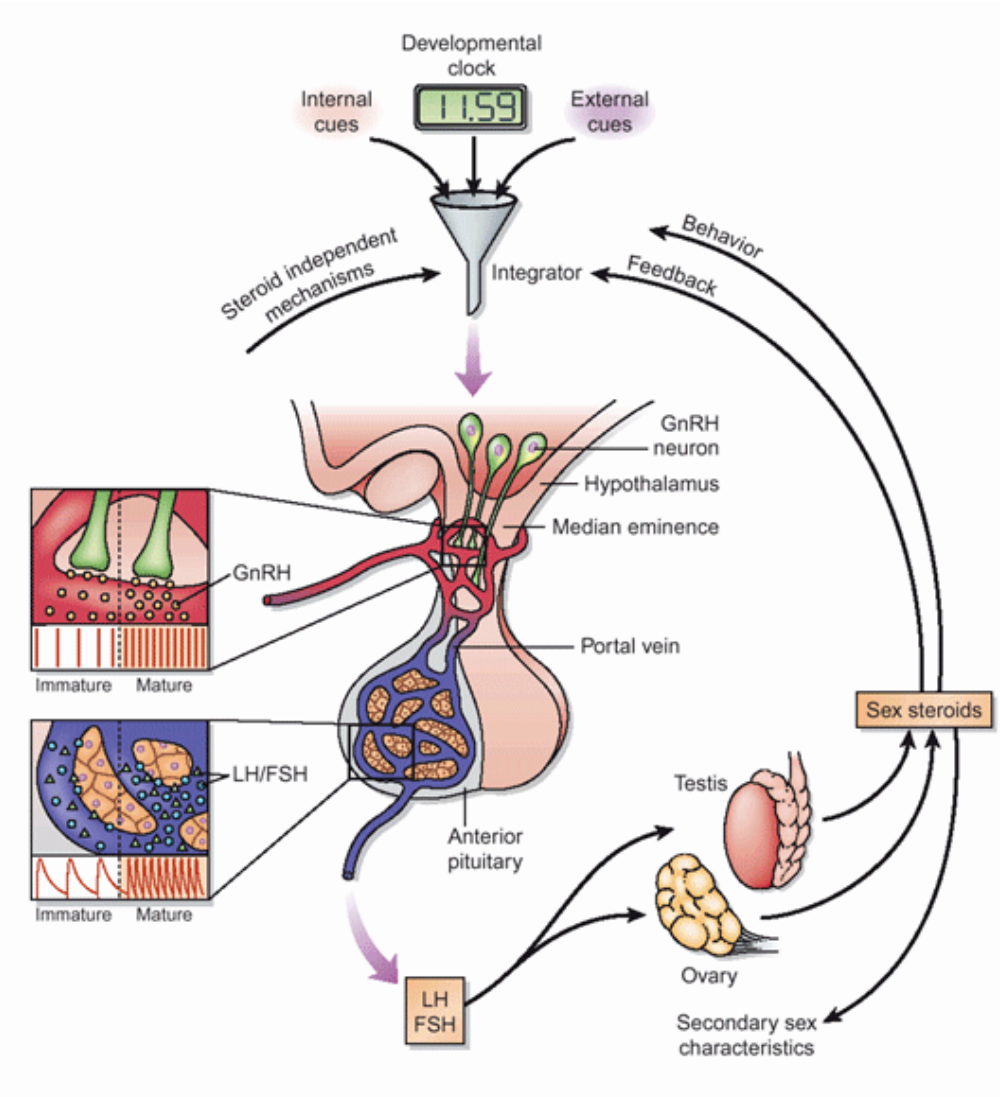

FIG.2: Pathogenesis of the pubertal process. Possible causes of reactivation of GnRH, the blood-borne and the secretion of LH and FSH at gonads. Inhibitory feedback to the HPG axis (9).

Precocious and Early Puberty:

Precocious Puberty (PP) is the appearance of secondary sexual characteristics to a non-physiological age, which is accepted before age 8 in girls and before age 9 in boys in a Caucasian population in developed countries (± 2,5 SD respect the mean age). It's important to know that the age of onset of puberty has been advancing significantly in the recent years, partly because of contaminants environmental estrogenic action, with a self-limiting secular trend as it has been found in some countries of Eastern Europe(3,10,11).

A limit situation and an important one on this study is the **"Early Puberty" (EP),** would correspond with the onset of pubertal development between 8-to-9 years in girls and 9-to-10 in boys. This picture, although not strictly can be considered pathological, may have negative impact on the final height or social considerations, and therapeutic management is sometimes similar to true precocious puberty (PP)(10).

The limits about puberty age, have been really discussed during the last decade, for the publishing of some articles that advanced the age of the onset puberty(12), but finally those studies have shown that the methods of diagnosis were not the corrects: they used the inspection and not the palpation to estimate the pubertal study(2), so actually we continued with the criteria mentioned previously. Even so, the definition of appropriate age limits is crucial to restrict diagnostic evaluation and possible therapeutic intervention with abnormal precocious development(2).

Incidence

Scientifically sound epidiomiological data of PP are not available in the literature. According to the Asociación Española de Pediatría (AEPED), the incidence of the precocious puberty is calculate about 1/5000-1/10000 people, with a female/male ratio of 20:1(10,11).
In an epidemiological study Danish done between 1993 and 2001, with girls and boys between 0-9 and 0-10 respectively, shows the following results: prevalence around 0,2% in girls and less than 0,05% in boys. The incidence in 0-9 years old girls where around 0,5-8 cases/10.000 people in one year, and the boys between 0-10 years, between 1-2/10.000 people/year(13).

In the other side, EP prevalence is more difficult to specify, but using some cross-sectional studies published recently, we could calculate that 2% of the girls between 8-9 years present EP(14).

Types and aetiology:

It is important to differentiate between **central precocious puberty (CPP)** and the **peripheral precocious puberty (PPP)** types of PP and EP because of the differences in differential aetiologies and diagnoses (TABLES 1 and 2) and because of the fundamentally different treatment options(11). Some authors also differentiate if the central or the peripheral are transient or permanent and they give importance to this date, because the recognition of transient forms, at least 50% of the precocious puberty(15), is of particular importance in order not to initiate unnecessary treatment in these patients(11).

So we can find:

- **CPP (gonadotrophin-dependent, "true")**(11): it is produced by a premature activation of the GnRH and consequently activating FSH and LH. The 98% of the precocious and early puberty correspond to CPP and the 58%-96% of this CPP seems belong to the idiophatic form, which risk factors we will explain below(2,10,11). A recent Spanish study published that the global prevalence of PPC in Spain is about 0,00037 in girls with an incidence of 0,13-2,17 cases/100.000/year(16).

  Central precocious puberty does not present as a homogeneous clinical picture, but is much more a continuum of clinical presentation and rate of progression ranging from slowly progressive or transient forms to rapidly progressive forms(11).

- **PPP (gonadotrophin-independent, "pseudopuberty"):** it is mediated by the extragonadal secretion of the sexual steroids, so HPG is not activated and the GnRH levels are normal. Some authors describe it with a prevalence of 2% but others conclude that there aren't general epidemiological studies about it(10,17).

- **Combined PP**: it is when the activation of the HPG axis is produced after the stymul of any peripheral cause(10). This one is really exceptional, and normally it is caused after a long PPP evolution that after receive therapy stopped sharply the negative feedback of the sexual steroids, so it actives the HPG axis. In other causes, it can be observed and up-regulation phenomenal which consists in a continual feedback of the gonadal axis, which is produced by big steroids quantities that produced an hyphothalam-hyphofisary activation that causes an increase of the gonadotrophines secretation, and finally a central activation of the puberty(18).

There exists some variants of the normal pubertal development, which defined the secondary characters onset in an isolated and early form. These changes can remain stables or even return, to present a true puberty in a normal form and time or, in the opposite, progress to a true PP(10):

- **Premature adrenarche/pubarche** is the increase of pubertal adrenal androgens. It may be seen in both sexes in normal children between 6-8 years of age. It occurs as a result of increased secretion of androgens from zona reticularis from the adrenal cortex. So this mechanism is different of the PP and EP one. However, there is a risk for early menarche in some cases with onset of pubarche between 7-8 years. The predicted adult height is also affected in some cases. During the diagnosis it is important reject an increase of 17-hidroxiprogesterone (17-OP-P), testosterone or Δ4-androstenodione, which could meant a pathological adrenal or gonadal secretion(19).

- **Precocious Thelarche Isolated.** Premature thelarche typically starts before 2 years of age and breast development is isolated. It may be unilateral. Somatic development or either bone age are not advanced. The situation regresses by time.

  The follow-up for PP is necessary in cases that were defined as thelarche variant. Premature thelarche which starts after 2 years of age may progress to a peripheral PP or EP or just regres after a time. Another point which should be kept in mind is, that some of the premature thelarche cases are due to exposition of estrogenic environmental pollution.

TABLE 1. Aetiology of central precocious puberty (gonadrotrophin-dependent, "true") (11)

Category	Underlying disease
Permanent precocious puberty	
Idiopathic	Sporadic
	Familial
CNS abnormalities or lesions	Hypothalamic hamartoma
	Tumours: astrocytoma, craniopharyngioma, ependymoma, glioma, LH-secreting adenoma, pinealoma
	Congenital malformations: arachnoid cyst, suprasellar cyst, phakomatosis, hydrocephalus (± spina bifida), septo-optic dysplasia
	Acquired disease: inflammatory CNS disease, abscess, radiation, chemotherapy, trauma
Dysmorphic syndromes	Williams-Beuren syndrome
	Klinefelter syndrome (rare)
CNS maturation with central precocious puberty secondary to prolonged sex steroid exposure:	Congenital adrenal hyperplasia
	Sex steroid-producing tumours
	Male-limited precocious puberty (constitutively activated LH receptor)
Transient precocious puberty	Idiopathic sporadic
	Arachnoid cyst
	Hydrocephalus
Variants of pubertal development (partial or incomplete precocity)	Premature thelarche
	Premature pubarche
	Premature menarche

TABLE 2. Aetiology of peripheral precocious puberty (gonadrotrophin-independent, "pseudopuberty") (11)

Category	Underlying disease
Ovarian disorders	Granulosa cell tumour
	Theca cell tumour
	Other oestrogen-secreting tumours: teratoma, teratocarcinoma, dysgerminoma, luteoma, mixed cell tumour, lipoid tumour
	Sex-cord or Sertoli-cell tumour of the ovary with annular tubuli seminiferi (SCTAT) and aromatase activity in Peutz-Jeghers syndrome
	McCune-Albright syndrome (ovarian cysts)
	Autonomous isolated ovarian cysts
Testicular disorders	Leydig cell adenoma
	Constitutively activating LH receptor mutation (male-limited precocious puberty = testotoxicosis)
Adrenal disorders	Adrenal adenoma
	Adrenal carcinoma (usually virilizing)
	Congenital adrenal hyperplasia (21-hydroxylase or 11β-hydroxylase deficiency)
HCG-secreting tumours	Dysgerminoma, teratoma, chorioepithelioma, choriocarcinoma, hepatoblastoma, pinealoma
Exogenous	Sex steroid exposure: pills (oestrogens; anabolics), food additives, cosmetics, creams etc.
Transient precocious puberty	Autonomous isolated ovarian cysts (self-limiting)
	Exogenous (interruption of exposure)

HCG = human chorionic gonadotrophin.

A real increased of the idiopathic aetiology during the last decade seems to be an important condition to be commented and analysed in background, especially for this project. To try to understand it, we think that it is important to set in some concepts we have cited previously which could affect on the onset of puberty: the genetic, metabolic, social and environmental factors.

So, in one hand, we have **genetic factors (family, ethnicity, gender).**

As we have comment before, it seems that around 74% of the variance involved in pubertal timing involved genetic effects and 26% environmental effects. About ethnicity, in the Breast and Cancer and Environment Research Centers study in this issue mean and median ages of breast stage 2 varied by it, and were 8.8 years for black, 9.2 for Hispanic, 9.6 and 9.7 for non-Hispanic, and 9.9 and 9.7 for Asian participants(20).

And on the other hand, new risk factors related with metabolic, social and environmental, have been announced in different studies which can explain this idiopathic course.

It has been proposed that since we are a fetus, the **intrauterine milieu** might influence physiological and pathological events occurring throughout life, also respect to puberty, where low birth weight appears to be associated with precocious pubarche in the humane female(2). In a study done in Spain, they

conclude that menarche ocourred on average 1.6 years earlier in low versus normal birth weight girls(21).

Another study demonstrated in Spain, following with Danish results(22), the **adoption** present a risk of develop CPP 25 times higher than the population born in Spain and the Spanish state adoption also increments the CPP(16).

A direct relationship between **body weight** and the age at onset of puberty was suggested in 1970, concluding that a critical amount of body fat as needed for the onset of puberty(2). Since then, several studies have treated this topic.

The Breast and Cancer and Environment Research Centers study finished in 2013 demonstrate that participants with BMI ≥85th percentile matured earlier than those <85%th percentile(20). In another study feeding juvenile female rhesus monkeys with a high-calorie diet results in an acceleration of body growth and precocious menarche. Serum leptin levels were stably elevated by high-calorie intake before precocious menarche. We speculate that leptin gives a signal to the GnRH neurosecretory system through GABA neurons. The stable, persistent elevations would provide an underlying excitable background for the activity of the GnRH neuronal system leading to puberty onset(23). But not only this mechanism influences the onset of pubertal development as well as tempo of puberty, in addition, adipose tissue has aromatase action, which increases androgen conversion to oestrogens. Adipose tissue is also related to increasing insulin resistance, which lowers sex hormone binding globulin levels and leads to increased bioavailability of sex steroids(24).

Althought the obesity epidemic is supported to be an important factor in the decline of the onset of puberty, factors involved with the new secular changes are far more complex. Thelarche occurred one year earlier in 2006 compared to 1991 in a study of 2095 girls in the Copenhaguen Puberty Study which was not explained by BMI or hormone levels, leading the researchers to postulate that other factors were involved(25).

Since then extensive interacting variables are become discovered to be associated with earlier development in addition to weight and genetics: certain intrauterine conditions and exposures, preschool high-meat diets, dairy products, low fiber intake, isoflavones, high-stress families, absent fathers, certain endocrine disruptors, the microbiome as it influences weight, epigenetics, light

exposure, hormone-laced hair products, insulin resistance, activity level, geographical location, and others. We will focus in the Endocrine Disrupting Chemicals (ECDs) in the next paragraphs.

The United States Environmental Protection Agency, defined an EDC as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostatis, reproduction, and developmental processes”. In fact, the Endocrine Society published a scientific statement in 2009 regarding EDCs and the evidence that they potentially impact many aspects of endocrinology including male and female reproductive organ formation and the HPG axis(25).

Theoretically, hormones or substances with hormone disrupting capability may interfere with pubertal development by actions at different levels, including the neuroendocrine HPG axis, the gonads and peripheral target organs such as breast, hair follicles and genitals. In the brain, EDCs may act by stimulation of oestrogen-sensitive nuclei including hypothalamic neurons thereby releasing kisspeptin and promoting a maturation of the hypothalamus causing EP or even PP, either peripheral with a direct ECDs effect or mature.

It is also possible that EDCs have direct effects on both body weight and the endocrine system of the HPG axis(26). The first group is known as “obesogens”, and they have an important rol at the obesity epidemic: as diethylstilbestrol (DES), bisphenol A (BPA), phthalates, perfluorooctanoic acid (PFOA), and organitins. But not all the ECDs act as obesogens(24).

Some studies of various potential EDCs show differing effects depending on the developmental period of exposure. There may be a “critical window” of susceptibility for each EDCs, which could interact with other EDCs, and impact the total body burden for an individual, depending on timing and dose of each exposure.
For instead, a study found that low dose prenatal and neonatal of EDCs increase in body weight of mice and this effect was seen by 6 weeks of life(24). And also neonatal exposure to phyto-oestrogens, BPA and oestradiol benzoate was correalated to early puberty in animals(26).

But, where we can found them?

For example, phthalates are found in toys, construction materials and clothing as higher molecular weight compounds and insolvents, cosmetics and pharmaceuticals in low molecular weight forms. Their

higher urine and serum levels in girls have been linked to both isolated early breast development as well as CPP in a study in Puerto Rico where the incidence of premature thelarche is the highest in the world(25).

BPA can be found in Tupperware, plastic bottles, food cans and medical products and at high temperatures escapes from its solid material into the containers contents. In a study published in Nature in 1999 demonstrated premature first estrus in mice that were born to mothers fed BPA.

But not all the ECDs are synthetics, some natural chemicals found in human and animal food can also act as endrocrine disruptors. A recent study reported that urinary concentrations of the phytoestrogens genistein and dadzein were 500-fold higher in infants fed soy formula compared with those fed cow's milk formula(27).

But, we have said, not only our HPG axis is altered by ECDs, the next figure (FIG.3) demonstrates that all hormone-sensitive physiological systems are vulnerable to EDCs, including brain and hypothalamic neuroendocrine systems, pituitary; thyroid; cardiovascular system; mammary gland; adipose tissue; ovary and uterus in females; and testes and prostate in males(27).

Nowadays, the United States Food and Drug Administration and the United States Environmental Protection Agency are in charge of controlling the risk of environmental substances, which is a significantly daunting task in light of how little is known regarding the effects of the numerous chemicals we are exposed to every day. In addition to the Endocrine Society, the European Society for Paediatric Endocrinology and the Paediatric Endocrine Society have also endorsed position statements calling for basic and clinical research, epidemiologic studies and the recognition of EDCs in clinical practice(25).

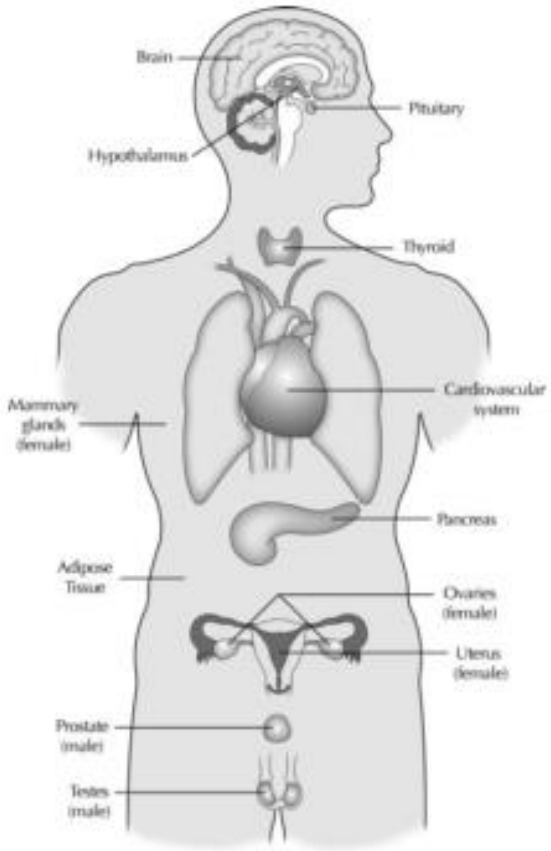

FIG.3: Systems vulnerables to ECDs. (27)

Evaluation

The first step in evaluating a child with PP and EP is the clinical history to obtain a complete family history (age at onset of puberty in parents and siblings and their respective highs) and personal history, including the possible time of onset of puberty and the progression of the pubertal manifestations, and if exists any evidence suggesting possible central nervous system dysfunction, such as headache, increased head circumference, visual impairment, or seizures. Growth should be measured, because progressive PP is almost invariably associated with a high growth velocity. In fact, a high growth velocity may also precede the onset of other pubertal manifestations (TABLE 3)(15).

The stage of pubertal development should be classified as described by Tanner (ANNEX1). At the past, we talked about the commonly used markers of the timing of female puberty were thelarche and menarche, but they both have some problems, the first one is not easly distinguished from fat tissue in slightly obese girls, and the second one is a relatively late marker of female puberty(2). In spite of that many paeditrician use the first method to evaluate patients.
Careful assessment is needed in obese girls to avoid overestimating breast development. A breast ultrasound could help us to differentiate it.

The development of pubic hair results from the effects of androgens. In girls, pubic hair in the absence of breast development is suggestive of adrenal disorders, premature pubarche, or exposure to androgens.

The physical examination should include an assessment for signs of specific causes of PP, such as hyperpigmented skin lesions suggesting neurofibromatosis or the McCune– Albright syndrome.

Also, PP and EP changes have been associated with high levels of anxiety in girls, and psychological evaluation may be useful(15).

Although no evidence-based algorithm is available to guide testing, evaluation of the mechanism and potential for progression of PP is generally recommended in girls who have precocious breast development at stage 3 or higher or at stage 2 with additional criteria such as increased growth velocity or who have symptoms or signs suggestive of central nervous system dysfunction or of peripheral precocious puberty(15).

Even so, the AEPED provides us of a possible evaluation algorithm (FIG.3).

TABLE 3. Criteria for Differentiating Progressive from Nonprogressive Forms of PP and EP. (15)

Criterion	Progressive Central Precocious Puberty	Nonprogressive Precocious Puberty
Clinical		
Progression through pubertal stages	Progression from one stage to the next in 3–6 mo	Stabilization or regression of pubertal signs
Growth velocity	Accelerated (> about 6 cm per yr)	Usually normal for age
Bone age	Usually advanced by at least 1 yr	Usually within 1 yr of chronologic age
Predicted adult height	Below target height range or declining on serial determinations	Within target height range
Uterine development†		
Pelvic ultrasound scan	Uterine volume >2.0 ml or length >34 mm, pear-shaped uterus, endometrial thickening (endometrial echo)	Uterine volume ≤2.0 ml or length ≤34 mm; prepubertal, tubular-shaped uterus
Hormone levels		
Estradiol	Usually measurable estradiol level with advancing pubertal development	Estradiol not detectable or close to the detection limit
LH peak after GnRH or GnRH agonist‡	In the pubertal range	In the prepubertal range

Bone Age

Although at this point we know physically our patient and her chronologic age of, some parameters give us more information about their maturation age: the effect of sex steroids on epiphyseal maturation. There exists some reference atlas which can be useful to evaluate them.

In true PP and EP, bone age is usually accelerated by more than two standard deviations to the chronological. Notably two exceptions to this "rule": when the PP mediated by gonadotropins is associated with a deficit of GH, can bone age in that case be very variable, and when associated with hypothyroidism, which presents with delayed bone age.

Bone age can also be used to predict adult height, although the precision is low (with a 95% confidence interval of about 6 cm below to 6 cm above the predicted value), and predictions tend to overestimate adult height.

Pelvic Ultrasonography Scans

In girls, pelvic ultrasound (PU) can reveal some PPP causes as ovarian cysts or tumors. On the other hand, uterine and ovarian changes due to oestrogen exposure can be used as an index of progressive puberty. A uterine volume greater than 2.0 ml has been reported to have 89% sensitivity and specificity for PP(15). The ovaric length (>3cm in pubertal phase) and the relation between body/neck uterins, are larger at puberty (approximately 1:1 and 2:1). Ovarian volume than 4-4,5 cc^3 is correlated with a puberty clinically demonstrated, because their grown is only gonadotrophines dependent.
The existence of microcysts (diameter <9 mm) is not specific to ovarian pubertal change found in 53% of normal prepubertal girls and 63% of early puberties; apart is the case of larger cysts with associated asymmetries give in ovarian volume, are highly suggestive of peripheral PP (cysts autonomous prefectures, McCune Albright syndrome, etc.)(10).

Hormonal Measurements:

Gonadotropin determinations (based on ultrasensitive assays) are central to the diagnosis. The gold standard for evaluation is the measurement of gonadotropins after stimulation by GnRH or a GnRH-releasing hormone agonist (GnRH test). Peak luteinizing hormone levels of 5 to 8 IU per liter suggest progressive CPP.
Caution should be used when interpreting gonadotropin levels in children younger than 2 or 3 years old, because gonadotropin levels are normally high in this age group. Random measurements of follicle-stimulating hormone are not useful, since they vary little throughout pubertal development(15).

Brain Magnetic Resonance Imaging

In all cases we suspect a possible hypothalamic lesion; magnetic resonance imaging (MRI) of the brain should be performed to determine it(15).
Finally, note that in the case of McCune-Albright syndrome, a scintigraphy with Tc-99 is more sensitive and safer than the traditional "bone series" for diagnosing, of earlier mode, fibrous dysplasia polyostotic feature this entity.

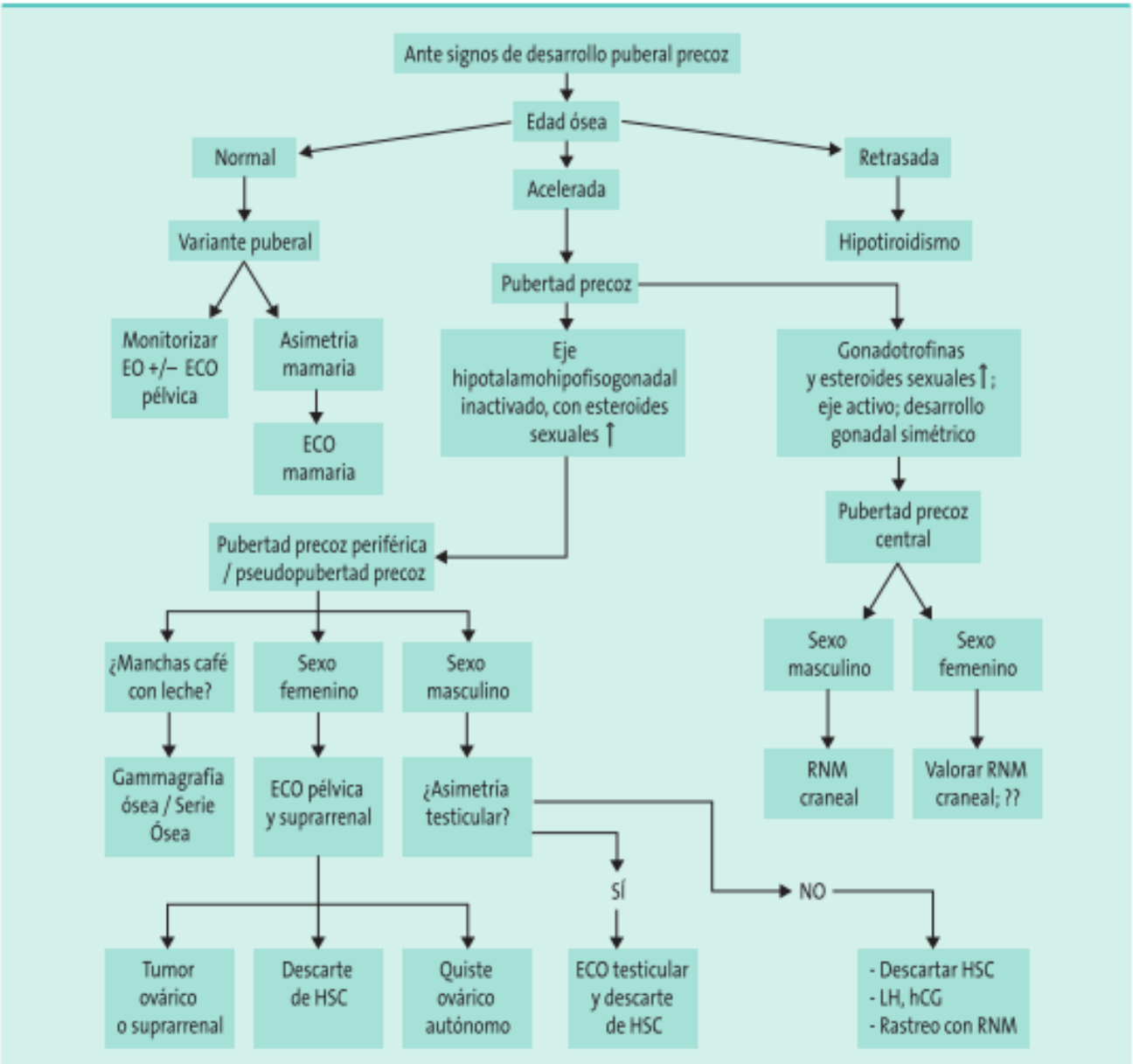

FIG.4: Evaluation algorithm from AEPED. (10).

Management

The treatment of PP and EP should be aimed at resolving the underlying cause premature ripening HPG axis. Establish the aetiology should be priority in all patients.

Peripheral Precocious Puberty:

Surgery is indicated for gonadal tumors.

If there is exposure to exogenous sex steroids, it should be withdrawn.

Aromatase inhibitors have been used to inhibit the production of oestrogens, and selective oestrogen-receptor modulators have been used to interfere with the action of oestrogens in the McCune–Albright syndrome.

Central Precocious Puberty:

GnRH agonists are indicated in progressive CPP. They work by providing continuous stimulation of the pituitary gonadotrophs, leading to desensitization and decreases in the release of luteinizing hormone and, lesser extent, follicle-stimulating hormone. Several GnRH agonists are available in depot forms. In open-label, noncomparative, longitudinal studies, the use of GnRH agonists consistently resulted in the regression or stabilization of pubertal symptoms (TABLE 4).

For cases in which precocious puberty is caused by a central lesion (e.g., a mass or malformation), management of the causal lesion generally has no effect on the course of pubertal development. Hypothalamic hamartomas should not be removed surgically for the purpose of managing precocious puberty. When precocious puberty is associated with the presence of a hypothalamic lesion, there may be progression to gonadotropin deficiency(15).

Notice that although the EP nor is strictly a pathology, it can have negative repercutiation in the height or in social therms, so their management could be sometimes similar to PP(10).

TABLE 4. Criterial for the GnRH use in the idiophatic CPP(10).

Edad • Niñas: edad de inicio de desarrollo mamario menor de ocho años (menor de siete años en niñas de origen mediterráneo) o menarquia antes de los nueve años • Niños: tamaño testicular > 4 ml antes de los nueve años
Ritmo de progresión • Rápidamente progresivas al diagnóstico: relación maduración ósea/edad cronológica > 1,2
Pronóstico de talla final • Talla pronosticada al diagnóstico inferior al percentil 3 de la población de referencia • Reducción de la talla pronosticada en 5 cm en cualquier momento del seguimiento
Factores psicosociales

Consequences of PP and EP:

One consequence that concern families is a possible esthetical problem about height; several studies have assessed adult height in people with a history of precocious puberty. In older published series of untreated patients, mean heights ranged from 151 to 156 cm in boys and from 150 to 154 cm in girls, corresponding to a loss of about 20 cm in boys and 12 cm in girls as compared with normal adult height. Height loss due to PP is inversely correlated with the age at the onset of puberty, and today, treated patients tend to have a later onset of puberty than did patients in historical series(28).
Nevertheless, in a longitudinal study do it in Barcelona, provides that genetic factors, and no the time when pubertal growth begins, influenced adult height. Although the duration of postnatal growth is shorter in earlier than in later matures, the former gain more centimetres during pubertal growth and reach similar adult height(29).

In addition, early pubertal timing associates with mental health problems in middle adolescence(30), particularly in girls with emotional and behavioural problems and early sexual activity(31). Maturing earlier than peers seems to create additional stress in the process of adapting to the changes in one's own body and social role brought about by pubertal maturation(30).

In the other hand, early menarche is characterized by excess body fatness and insulin beginning in early childhood and higher prevalence of clustering of adverse levels of risk variables of metabolic Syndrome X in young adulthood(32). Furthermore, a cohort study provided evidence that early age ofmenarche is associated with increased risks of CVD events, CVD mortality, and overall mortality in women, and these associations may be only partly mediated by increased adiposity. History of early menarche (<12 years old) may help to identify women with increased risk of CVD and mortality(33).

Early environmental exposures that mimic oestrogens, may be related to earlier breast development, thereby putting the rapidly proliferating mammary tissue at risk. Pubertal girls who are exposed to carcinogens in the presence of a changing hormonal profile may be at high risk as a resultof susceptibility of the mammary epithelial cells to early insults and mutations that make them vulnerable to carcinogenesis(34).

Elx city

Another point to be introduced is Elx, which is the city of our study and it is important to know it to understand why we are performing this project.

Elx, or Elche, is a city located in the county of Baix Vinalopó, Spain. According to the 2014 census, Elche has a population of some 228,647 inhabitants, ranking as the third most populated city in the Valèncian Community (after València and Alicante) and the 20th largest Spanish city.

Part of the municipality is coastal but the main city is some 11 km (6.8 mi) from the Mediterranean Sea. A small creek called Vinalopó flows through the city splitting it in two parts.

The economy of Elche is based, in large part, on the footwear industry, with over 1,000 shoe factories, being one of the most important footwear centres in Spain and the rest of Europe with brands like Pura Lopez or Panama Jack.

There are other economic activities in Elche: agriculture (dates, olives, cereals and pomegranates), although it has lost importance in recent years; rubber industry; trade, which employs 20% of the workforce; and tourism (35–37).

3.2 JUSTIFICATION

We have several reasons to be interested to perform this study.

The first reason is to try to get an objective answer for our subjective perception that in Elx exists a higher PP and EP prevalence than in other cities, notified by the main Endocrinologist Paediatrician of Hospital General Universitari d'Elx (HUE) who has been working in other cities as València or Barcelona.

During the last decade, many articles and studies are alerting on the seculars trends and greater incidence of PP on EP(2,12). But any of these epidemiological studies have used a representative sample to calculate a real prevalence as we want to do(11), going to schools to find any girl with risk to develop PP or EP. Thus maybe we are talking about an underdiagnosed disease. We will perform the first study in this area.

It is important to notice that in this way, in Spain, a research group called PUBER published a study talking about the incidence and prevalence of CPP(16), which can have really interest in our endocrinal world, but it has the same limitation: investigators only used the patients who came to their clinicals, letting those who hasn't realise that the earlier onset of puberty could be pathological.

We suspect that this possible higher prevalence could be explained by the footwear industry and the materials they used, as plastics and adhesive, which could be playing a ECDs role on the population who has have been in contact with this materials (2,11,24,26,27). Not only because the individual exposition, there exists the knowledge that these footwear workers could take this product to their houses to work there. Consequently the exposition can affect other family members, causing a possible concern public health problem. But no longer, the Paediatric department has this opinion, sundry physicians on HUE has shared with us the same vision. For example plastic-surgeons have notified more breast reduction surgeries than in other hospitals.
The reason to choose València such as our "control group" to compare the PP and EP prevalence is because sharing the same Community offers us facilities logistics and similar live conditions which can affect to the puberty process, such as geographic location or light situation(2). But, with an important contrast: València has not a footwear industry as Elx.

Therefore, this study will be the first step in a long pathway to discover if our perceptions are true: trying to find a higher PP and EP prevalence explained by a possible footwear industry exposure

compared with girls from València, who are not. In fact, United States Environmental Protection Agency and other institutions alert of the ECDs effects and encouraged the research of it(27).

On the other side, to fill a questionnaire with some of risk factors already demonstrated, such as obesity, genetics, ethnic, adoption or low weight at birth (8,16,20,21) will allow us to review if other factors are affecting our population too.

As literature says, exogenous substances can affect PP and EP in a peripheral way, so, the fact to find by the PU, participants' ovaries in a prepubertal stage will help us to support ECDs hypothesis. Even so, as we have said previously, it seems that the most of the PP and EP are central, and a 95% of it is idiopathic, so maybe ECDs are related too and they are undervalued(2,10,11,17).

Precocious and Early Puberty could have many consequences in girls as we have commented before, such as esthetical, with lower height, mental and behavioural and maybe future ones as decrease of the menopause age (28,30–34). Find a clear higher prevalence in our city can alert paetricians and parents to the existence and importance of this pathology, to detect it and treated precociously. The affirmative association with footwear industry can alert to the importance of tried to avoid the contact with the population with major susceptibility, as pregnant or children.

For all these reasons and believing that the results will help us to know better one of the most important endocrinologist paediatrian pathologies, we are disposed to perform this three years study of the PP and EP prevalence.

4. HYPOTHESIS

Main hypothesis:

- **The prevalence of precocious puberty and early puberty in girls is higher in Elx than in València.**

Secondary hypotheses:

- The precocious and early puberty risk factors as IBM ≥90 percentile (to the reference population and sex), mother's age at menarche, ethnic, adoption and low weight at birth also affect girls in Elx and València.
- The contact with footwear industry is associated with precocious and early puberty.
- The contact with footwear industry is considered to affect in a peripheral way to de precocious and early puberty.

5. OBJECTIVES

Main Objective:

- **To calculate the prevalence of precocious and early puberty in girls 8-to-9 years at Elx city and to compare it with València.**

Secondary objectives:

- To determine if the following risk factors as BMI ≥90 percentile (to the reference population and sex), mother's age at menarche, ethnic, adoption and low weight at birth are associated with precocious and early puberty.
- To determine if the contact with the footwear industry is associated with precocious and early puberty.
- To classify in central or peripheral the precocious and the early puberty to relate it with exogenous causes using pelvic ultrasound and the GnRH test.

6. MATERIALS AND METHOD

6.1 STUDY DESIGN

The study was a school-based, cross-sectional study of pre- or early pubertal girls, selected by a two-stage sampling strategy.
Two centers from Comunitat Valènciana will take part in this study: Hospital General Universitari d'Elx (HUE) and the Hospital Universitari i Politècnic La Fe de València (HLaFe), specifically the Paedriatric Departments of both of them.

Data will be collected during an estimated period of 2 years to be able to establish the onset of puberty and to calculate and compare the prevalence of PP and EP in girls between Elx and València.

6.2 POPULATION

Altogether, 874 8- to 9- year-old girls, who attended schools and are living in the cities of Elx and València, Comunitat Valènciana, Spain, will be invited to participate in the study along with their parents or legal tutors.

6.3 INCLUSION AND EXCLUSION CRITERIA

INCLUSION:
All three criteria must be met:

- Girls with ≤9 years old
- Girls who studies in Elx city or València City.
- Girls living in Elx or València cities at least four years.

EXCLUSION:
At least one criteria must be met:

- Boys.
- Girls who are studying in Elx or València schools but they are not living at those cities.
- Girls with chronic diseases.
- Girls being treated with anabolic steroids or contraceptives.
- Those girls who their parents don't sign the informed consent.

6.4 SAMPLE

SAMPLING: We will do a probabilistic method sampling, using a cluster sampling procedure to select our participants. It will be also stratifed by city (Elx or València) and by types of schools by funding: private, public or states schools. This method has different steps:

1. We will choose each cluster using a non-probabilistic simple random method.
2. We will include all the girls of each cluster.

This is a bicentric study, in which two centres take part: HUE and HLaFe. The sample recruitment will take place in schools of both cities. Parents or legal tutors of candidate participants will be asked to participate and an information sheet describing the study will be given. After written informed consent is obtained from the girls' parents, these will be enrolled to participate.

SAMPLE SIZE:

The sample size and power calculator GRANMO was used. Accepting a alpha risk of 0,05 and a beta risk of 0.20 in a bilateral contrast, we will need 874 girls in València and other group of 874 girls in Elx to detect as a statistically significant the difference between both proportions. For València group we have expect the sum of the prevalences of PP and EP published at the different studies (10,13,14): 0,023 and for the Elx group we have expect a 0,05 of prevalence. A dropout rate of the 10% have being estimated too.

6.5 VARIABLES

Variables response: Precocious and Early Puberty in girls.

Girls with Precocious Puberty will be those ones who the appearance of secondary sexual characteristics to a non-physiological age, which is accepted before age 8(2).

Girls with Early Puberty will be those ones with the onset of pubertal development between 8-to-9 years(14).

We define the onset of the pubertal development as the palpation of the telarche, which is the first appearance of breast defined as Tanner B2 Stage(2,3).

Variable explanatory A: The City

We will define this variable as to living in Elx City or València City at least four years.

Variable explanatory B: Contact with Footwear Industry

We will define this variable as having any member of the family working in the footwear industry nowadays and living in the same house that the participant. The work has to be executed in a footwear factory for the previous four years.

This variable is chosen to demonstrate if footwear industry can be playing ECDs role.

Co variables:

Some of these variables are possible risk factors that may affect on the girls' onset of pubertal development (8,16,20,21)

- Age of the Participant: years
- Body Mass Index: kilos/metre
- Ethnic Group: Caucasian, Black, Asian, Amerindian (population living in North or South America before the Europeans arrived)
- Mother's Age at Menarche: years
- Lower Weight at Birth: we define low weight at birth as less than 2500 gr. This variable will be expressed in yes/no
- Adopted: yes/no

TABLE 5. Summary of Variables:

	Variables	**Description**
Response Variable	**Precocious Puberty and Early Puberty**	Categorical qualitative variable.
Explanatory Variables	**The City**	Categorical qualitative variable.
	Contact with Footwear Industry	Categorical qualitative variable.
Co variables	Participant's Age	Discrete quantitative variable
	Menarche's Age at Menarche	Discrete quantitative variable
	Body Mass Index	Discrete quantitative variable
	Ethnic Group	Categorical qualitative variable.
	Lower Weight at Birth	Categorical qualitative variable.
	Adoption	Categorical qualitative variable.

6.6 METHODS OF DATA COLLECTION AND STUDY CIRCUIT

a) Data collection

First of all, we will inform the rest of the members of the Paediatric Department from the HUE, including our nurses, about the study that is being carried out. Special requirement will be asked to the radiological department due the fact they represent one of the most important parts of the study concerning the pelvic ultrasound (PU) and providing a portable ultrasound.

We will do a personal meeting in the HLaFe to inform also the entire Paediatric Department including the Radiology Department.

A short pilot study proving the different stages of our study as breast palpation, questionnaire and the different tests will take place with some of our PP and EP patients.

Using our cluster sampling we will recruit from some schools where 8 years old girls and meeting the inclusion and exclusion criteria of the study will be invited to participate in our study.

Our first step will be to get the school approval and parents or legal tutors consent, so we will deliver the information document and informed consent to be signed (ANNEX 3 and 4).

It is important to note that our participants will be girls under-age and under 12 years old, so it is really important the parents approval. In fact, we have planted different meetings with parents of each school to inform about the study and to explain the importance of carry it out. With the same line and importance, participant's assessment will be really considered too.

b) Determinate PP and EP.

Our main Endocrinologist Paeditrian, previously trained, will go to the different classes of the same grade school (primary 3th grade) at both cities, Elx and València, to determine if the girls are starting puberty. In the selection visit, the inclusion and exclusion criteria and the inform consent signed will be checked.

The exploration method we have proposed to use to diagnose if the girl has or has not PP or EP is breast palpation. Breast palpation will inform us about thelarche. In case girls present adipomasty, we will use a portable ultrasound to determinate if exists the gland mammary(2,15).

We will go at the months of January and February, so we could diagnose those girls who could present PP, being that the most of them would not have turned 9 years yet.

In a second time, one year later, in the same months, we will come back to the same schools and we will repeat the same procedure. These girls will be between 8 and 9 years, so we will be able to diagnose EP. We will invite the girls with precocious and early thelarche to continue our study and we will inform the parents too.

The next step will be to cite these girls in the clinic at both hospitals of reference. This part will be two parts:

- Fill out a questionnaire, with some risk factors as the mother's age menarche, the ethnic group, measuring the BMI, low weight at birth and asking for adoption. We will use this questionnaire to ask if the girls have or not any contact with the footwear industry. Our investigators will explain parents and participants the meaning of each criteria (ANNEX 2).
- To determinate, through a PU and by a radiologist, if the girl have CPP or PPP. We will use the ovarian volume: a volume greater than 4 cc^3, we will classify as central. In case this would be under 3 cc^3, we will classify it as peripheral. This is because, as we have exposed previously, the ovaries are only gonadotrophines dependent(10). In the cases the volum would be in a borderline stage (between 3cc^3 and 4cc^3), we will use a GnRH hormone test to classify if the HPG axis is activated or stymulus stem from exogenous cause(15).

c) Filling the database and observe evolution.

We will fill our database and we will calculate prevalence of PP and EP in each city. We will classify our participants thought the questionnaire answers and the PU.

A summary of the protocol is presentated below (FIG.5)

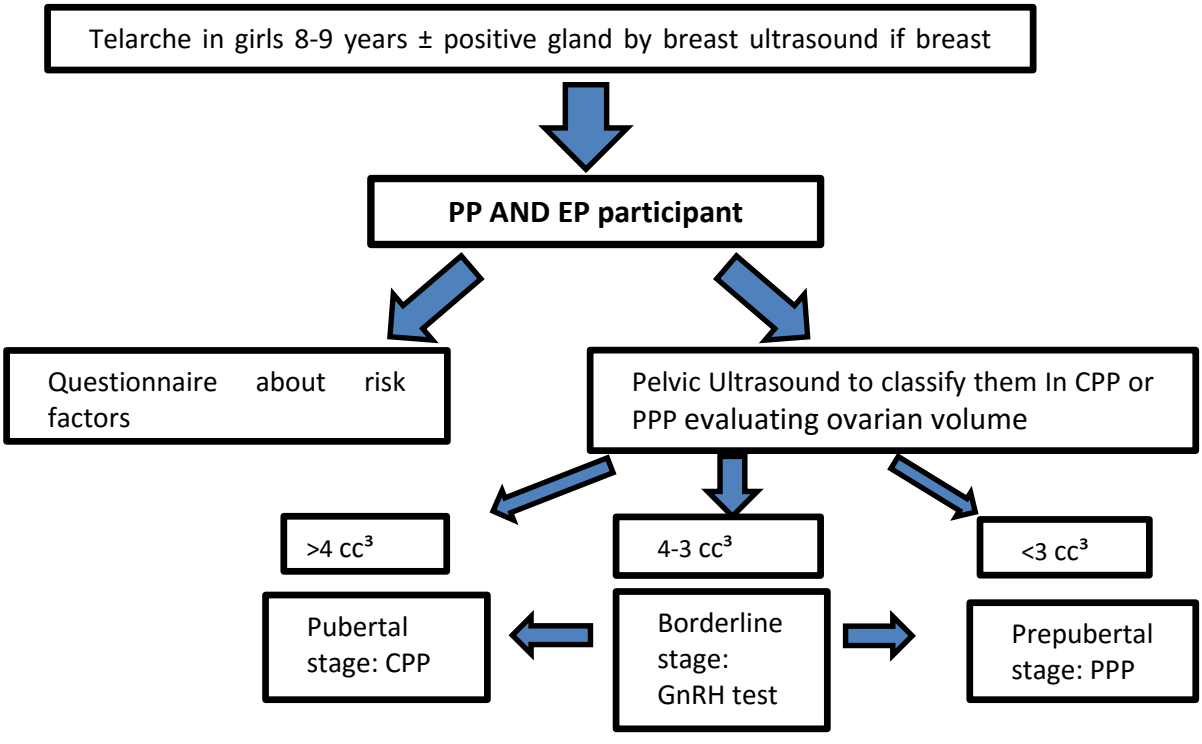

FIG.5: Summary of the study protocol

7. STATISTICAL ANALYSIS

7.1 DESCRIPTIVE ANALYSIS

It is important to note that in our study we cannot define and independent and dependent variable due the project is a descriptive cross-sectional study. Nevertheless, as our response variable, precocous and early puberty is qualitative or categorical, and our explanatory variables, the city and the contact with footwear industry, too the results will be expressed as percentages. In the same way, we will measured our qualitative or categorical co variables as ethnic group and low weight at birth and the results will be expressed as percentages too.

For the age, menarche's mother age and the BMI we will measured them as quantitative and results will be expressed by quartiles. Thus, assuming that they are not normally distributed, median will be estimated. In case that they follow a normal distribution, arithmetic mean and typical deviation will be calculated.

7.2 BIVARIATE ANALYSIS.

Percentages for categorical variables will be shown in a contingency table and chi-square test (χ_2) will be used to compare.
We will calculate too Odds Ratio and their confidence interval.
We will analyse this percentage for the explicative variable through contrast tables.
Stratified by the co variables (continuous variables will be categorized in quartiles).

7.3 MULTIVARIATE ANALYSIS

A multivariate logistic regression will be performed, taking our response variable PP and EP, and the explanatory variables, city and contact with footwear industry. We will adjust in all of the cases the co variables, to avoid portential confounders and/or effect modifiers (continuous variables will be categorized in quartiles).
To perform this analysis, we will use the IBM SPSS Statistics 22.0 program. Results will be presented with a confidence interval of 95%.

8. ETHICAL ASPECTS

Research Ethics Committee of each participating center will be evaluated and submitted for consideration, guidance and approval the study. Schools, parents or legal tutors and the participants will be informed personally by the researches and an inform document about the study will be given to them. Before being included in the project, participants' parents or legal tutors will sign voluntary the informed consent to participate in the study, according to *"Ley 41/2002 Básica reguladora de la autonomía del paciente y de derechos y obligaciones en materiade información y documentación clínica"* and the assessment of the girls. This informed consent will include all the posibles procedures girls need to do.

The information and dates of our patients and families will be confidential and only used for the project, according with *"Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal".* All data will be anonymously analysed. This information will be transmitted and guaranteed to girls and parents or legal tutors.

Our study will be carried out according to the ethical principles established in the Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects.

If we need to do an ethical reflection about our study, it is important to note the importance of a study with children, especially those who could be starting puberty.
First of all, the breast palpation could be an uncomfortable moment for girls, who will be respectfully explored, guarantying their personal intimacy. In the other hand, we project our study in the least harmful way for these children, trying to use the most innocuous procedures, as the PU, and only using those which could be more aggressive, such as GnRH test, in as few cases as possible.
Finally, as physicians, we guarantee that those girls who will be diagnosed with PP and EP will be included in a special group for a follow-up.

9. LIMITATIONS AND STRENGHTS OF THE STUDY

Analysing our study, several limitations have been detected and taking account to minimize the interference they may cause in our project.

1. Our study is looking to have a representative sample of the population of girls between 8-9 years old from Elx and València, for this reason we determine PP and EP at schools and stratified them thought the financing. However, it is important taking account to point at this selection phase:
    a. We are talking about healthy volunteer children, so we can find a non-response bias, where parents or aren't interested in take part in our study or during the time we loss them. This possible limitation can be settled with the different personal meetings we will have with parents at schools, the informed documentary and calling them to guarantee the following during the data collection. By the way, in our statistical analysis expect a losses about 10%, so we contemplate possible losses to follow up.
    b. It is possible that some schools don't allow us to explore telarche in their girls. To minimize this limitation, we will meet personal of schools to explain the importance of our study and the minimal intervention we will do with the girls.
2. To determinate all the parameters we need to carry out our study, we need to train our different investigators, such as the residents or the nurses, to minimize the detection bias we can experiment. In the same way, the decision of only one physician measuring thelarche with the same criteria for all the participants is taken to avoid this possible limitation.
3. Our project is a cross-sectional study, so it is important to take account that we cannot analyses causality of PP and EP with the footwear industry contact, instead of we will determinate association, arising the possibility of future studies with this objective. At those future studies we will compare a case group with PP and EP and with footwear industry contact and a control group without PP and EP but with footwear industry contact.

10. WORK PLAN

10.1 TASKS AND MEMBERS OF THE STUDY

The research team is formed by a multidisciplinary equipment of paediatrics physicians, paediatrics residents of at least 2n year, 4 radiologists, 4 paediatrics nurses.

- The main researcher (MR): It will be in charge of the whole coordination, financial management and publication and diffusion.
- Endocrinologist Paediatrics Physicians (2) (EPP1/EPP2): One of each hospital. Endocrinologist Paediatric from Elx (EPP1), will be in charge of the exploration of all the girls. The other one in València (EPP2) will help him/her to coordinate the project in Hospital of València.
- Radiology Physicians (4)(RP1, RP2, RP3, RP4): Two in each hospital. They will be in charge to do the ovarian Radiology to the girls, measuring the ovarian volume.
- Analytics Physician (AP): He will collaborate in the drafting of the results interpretations and conclusions of the study.
- Paediatric residents (4)(PR1, PR2, PR3, PR4): Two for each hospital. At the different cities and previously trained, they will help the physician carried of the exploration with the breast eco with those girls who was needed, results, informing the parents and helping at the consult office filling the questionnaires
- Nurses (4) (N1, N2, N3, N4): Two for each hospital. They will be on charge to cite participants to the clinical and to measure BMI in each patient and do the GnRH tests.

10.2 STUDY PHASES

The study has been divided in four phases:

1. **Preparation and coordination phase (1 year):** in this stage a first meeting of the research team will be performed to explain clearly the objects of the study and establish all the steps of the study that must be performed in both hospitals: main researcher and the endocrinologist paediatric from Elx will travel to Hospital Universitari la Fe de València to give the same information. A chronogram will be given to each member of the research team to make work easy and also all tasks will be extensively defined. Furthermore, a meeting and some session review will be done with the rest of both Paediatrics departments to make it understanding and to ask for the collaboration of all the physicians.
   At this point, problem identification, suggestions from all the physicians, and final elaboration and evaluation of the research protocol will be carried out.

   The protocol will be send to the CEIC of HUE and HLaFe. All the recommendations will be considered and the study won't start until the CEIC approvals will be received.

   In this phase, residents will be trained to be able to do breast ultrasound by radiologist and to fill in the questionnaires. Nurses training to know how calibrate instruments, measure BMI and do GnRH test will take place too. Before the definitive data collection and the study setting up, a short pilot study will be undertaken with some PP or EP patients of the Hospital of Elx who previously have accepted, to correct or improve possible complications or deficiencies from the procedures.
   A procedure manual will be redacted to help the team or collaboration if any doubt exists during any phase.

   Besides, the data base will be created by the statistician.

   During this phase, schools will be chosen and meetings with schools personal and parents will be done. The inform documentary and the informed consent need to be signed in this phase.

2. **Fieldwork and data collection (18 months):** During the months of January and February of two consecutive years, those girls who meet exclusion and inclusion criteria will be examined by the main endocrinologist paediatric and helped by the residents to diagnose PP and EP. A

coordination meeting after the first evaluation will take place to improve any problem the investigators may have had.

Parents or legal tutor and participants with a possible PP and EP will be cited by phone to our office consult to continue with the project.

PU, questionnaire, measure BMI and GnRH will be done by the investigators on this phase. These procedures will take place in two moments of the study: the first one after the first participants' evaluation, to those who possible have a positive onset of puberty (possible PP). And the second one, after revaluation, with those participants with a positive breast gland (possible EP).

Another cite with the parents to explain results will take place too.

Data will be collected and included on the database.

3. **Data analysis and final evaluation (4 months):** in this period the statistical consultant, the main researcher and the investigators will be in charge of analyse all the results and to write all results and conclusions in a final article. Every participant and parents will receive a postcard communicating research team's gratitude for their cooperation in the study.

4. **Publication and diffusion (2 months):** The main researcher will be in charge of the final publication of the study and, together with some of the rest of the investigators team, to perform the diffusion of the results attending to conferences.

10.3 CHRONOGRAM

	2015	2016						2017						2018						Personal
TASKS	N-D	E-F	M-A	M-J	J-A	S-O	N-D	E-F	M-A	M-J	J-A	S-O	N-D	E-F	M-A	M-J	J-A	S-O	N-D	
Prepare the protocol and research team coordination																				MR, EPP
CEIC approval																				CEIC
Diffusion of the protocol																				EPP, MR
Train physicians and fellows																				MR, EPP, RP
Database creation																				St
Pilot prove test																				EPP
Contact with the schools and parents																				EPP
Data collection																				PR, EPP
Patients selection																				PR
Protocol revaluation																				
Patients follow up and revaluation																				R, EPP, NN
Interpretation of the results																				MR, EPP1, PR
Preparation of the article																				MR, EPP1
Scientific publication																				MR, EPP1
Attend conferences																				MR, EPP1

11. FEASEABILITY

Altough it seems a really ambitious project, Endocrinologist Paediatrician, Radiologist, Residents and Nurses, together with the rest of the Paediatric Departments, of both Hospital has experience in other epidemological studies.

About the procedures, HUE and HLaFe provides of all the instruments and means to develop the project. The distance and the relation between both hospitals make the project available and more interesting.

Furthermore, the hospital will provide the informatics equipment suitable to processing database for the study development without additional cost.

12. BUDGET

We can analyse our budget in three sections:

a. **Personal expenses**: investigators will not been paid. After analyse the possible extra hour they could do, such the nurses or the radiologists, we assume that the study work is include in their assistance time. In the other side, residents will assume this work into their learning phase.

b. **Executive expenses**: we have some costs we need to count:
   - Ultrasound portable: a new ultrasound portable will be required for breast ultrasound on schools. Although the hospital have some, we will need one for develop the study.
   - Pelvic ultrasound: this procedure will be perform at the hospital and any additional expenses related.
   - GnRH test: this procedure will be financed for the study because it is not implement regularly.
   - Statiscian: it will be hired a statician to perform all the statistical analyses. 40 hours of work will be needed, paid 35€/hour.

c. **Travel and subsistence expenses**: all these expenses are destine to the time visiting schools. We count that the cost to travel to all the schools in Elx during two weeks could be 50€. Travel to València (50€) and the accommodation for two weeks (500€). Visit schools in València will cost 50€.

d. **Publication and dissemination expenses**: we have calculated some expenses for the creation of an open access article publication (2000 €) and to the attendance to scientific meetings and national congress (1000€ each one) to announce the study results.

Expenses		Costs
1. Personal expenses		0 €
2. Executive expenses		
Ultrasound portable		7000 €
Questionnaire		0 €
Pelvic ultrasound		0 €
GnRH test		300 €
Statistitian	40h x 35€	1400€
3. Travel and subsistence expenses		
Visit schools in Elx		100 €
Travel expenses to València		50 €
Accommodation in València two weeks		500 €
Visit schools in València		50 €
4. Publication and dissemination expenses		
Open access article publication		2000€
Attendance to scientific meetings and national congress.	2 x 1000€	2000€
TOTAL		**11400€**

13. HEALTHCARE IMPACT

To determine the prevalence of the PP and EP in Elx and also in València will possible have an impact in the health system because as an epidemiological study, it will allow us to know our population's pathologies better, this one with a high importance in the endocrinologist paediatric world.

If the results show that in Elx there is a higher prevalence than in other cities with València as reference, and it has association with footwear industry exposure, new prevention programs could be developed and it will force us to be more aware of a pathology that could be underdiagnosed. Detect and treatment the girls will allow us to reduce their consequences, not only on physical terms but also at a psychological level.

Finally, we think that is important to notice the relevance finding some ECDs association results with a disease which affect our own city population. Maybe this study is the first one to encourage people to arise news projects around this interesting topic, the Endocrine Disrupting Chemicals.

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15. ANNEXES

ANNEX 1. Tunner breast stages to diagnose puberty (3)

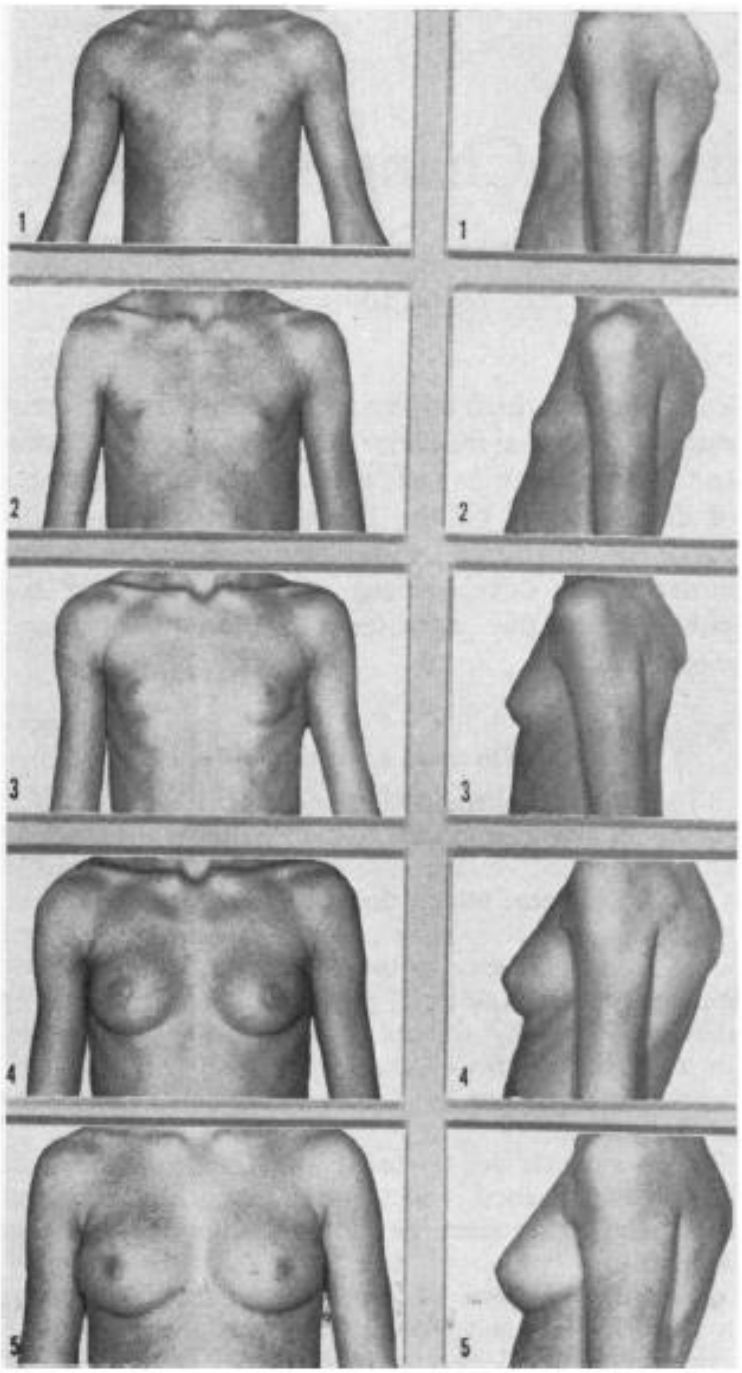

FIG. 1.—*Standards for breast ratings. (From Tanner, 1969.)*

ANNEX 2. Data collection sheet (to refill by the participant and clinical researchers)

PARTICIPANT CODE: _____

- **Date of birth:** ___ / ____/ ____
- **Date of first visit:** ___ **/** ____ **/** ____
- **Mothers Age Menarque (years):** _____
- **Ethnicity**: Caucasian ☐
  Black ☐
  Asian ☐
  Amerindian ☐
- **Height** (cm): ___,___ **Weight** (kg): ___,___
- **Body Mass Index** (percentile for the reference population and sex): _____
- **Adoption: Yes** ☐
  No ☐
- **Low weight to birth (less than 2.500): Yes** ☐
  No ☐
- **Possible contact with Footwear Industry: Yes** ☐
  No ☐
- **Result of the ovarical ecography (ovarical volum):**

_____
_____
_____
_____
_____

- **It was necessary any other test:**

_____
_____

ANNEX 3. Consent document

Hospital General Universitario de Elche Hospital Universitario y Politécnico La Fe de València	HOJA DE CONSENTIMIENTO	**Prevalencia de la Pubertad Precoz y Adelantada en niñas de Elche. ¿Realmente es tan alta?**

CONSENTIMIENTO POR ESCRITO DE LOS PADRES/TUTORES LEGALES:

TÍTULO DEL ESTUDIO: Prevalencia de la Pubertad Precoz y Adelantada en niñas de Elche. ¿Realmente estan alta?

Yo……
Como madre, padre o tutor de la niña……
Confirmo que:

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
Se han respondido mis preguntas de forma satisfactoria.
He recibido suficiente información sobre el estudio.

He hablado con (nombre del investigador/pediatra/enfermero):……..

Comprendo que la participación es volntaria, y que puedo retirarme del estudio cuando quiera, sin que eso repercuta en las curas médicas y sin dar explicaciines.

En consecuencia,

Doy mi conformidad para que mi hija participe en este estudio.

Sí ☐ No ☐

Doy mi conformidad para que se le realicen a mi hija las pruebas que los investigadores consideren necesarias:

Sí ☐ No ☐

Prometoque los resultados sean utilizados en investigaciones futuras relacionadas con la pubertad precoz y adelantada.

Sí ☐ No ☐

Firma del padre/madre/tutor de la participante:

Firma del investigador:

Fecha: __ __/ __ __/ __ __

Fecha: __ __/ __ __/ __ __

Versión 1_18.02.15

ANNEX 4. Inform document

HOJA DE INFORMACIÓN SOBRE EL ESTUDIO:

PREVALÈNCIA DE PUBERTAD PRECOZ Y ADELANTADA EN NIÑAS DE ELCHE. ¿REALMENTE ES TAN ALTA?

Código del Protocolo: **HUE-PEDPP-PE** Versión 1 final de fecha **18-01-16**

EudraCT: **2010-024414-61** Proyecto Ministerio Sanidad: **EC10-252**

El equipo de investigadores clínicos del Servicio de Pediatría del Hospital General Univerversitario de Elche, junto con la participación del Servicio de Pediatría del Hospital Universitario y Politécnico La Fe de Valencia, proponen la realización del estudio arriba mencionado, basado en observaciones propias y en trabajos científicos de investigación médica.

Introducción

La pubertad precoz y adelantada se define como un adelantamiento en edad del inicio del desarrollo pubertal, que implica la aparición prematura de los caracteres sexuales secundarios.

Consideramos pubertad precoz y adelantada a la aparición de telarquia (inicio del botón mamario) antes de los 8 años en chicas y un aumento del volumen testicular en chicos menores de 9 años. En cuanto a la adelantada, nos referimos a los mismos parámetros pero en chicas con límite en 9 años y en chicos en 10. La proporción entre sexo femenino y masculino de 20:1, hace recaer la atención principalmente en ellas. Estas edades, sin embargo, han sido objeto de múltiples proyectos en la última década poniendo en duda si deberían ser adelantados, debido a cambios sociales que subjetivamente se pensaban. Por otra parte, siempre se han considerado ciertas etiologías como las determinantes para la aparición de la enfermedad, ya sean de causa central o periférica, pero el alto porcentaje de pubertades precoces y adelantadas idiopáticas han alertado sobre otras posibles causas: como por ejemplo los llamados disruptores endocrinos, que son unas sustancias que interacción con múltiples sistemas de nuestro organismo, incluyendo las hormonas y que podemos encontrar en múltiples materiales de nuestro día a día, como maquillaje, botellas de plástico o en nuestro propio trabajo, con los pegamentos que pueden ser utilizados en la fábricación de calzado. El hecho de iniciar la pubertad y consecuentemente la menarquia antes de lo esperado, constituye un motivo de preocupación para familias y para los propios pacientes que pueden sentirse diferentes en un momento en el que todavía no se encuentren preparados.

Objetivo y realización del estudio

El estudio actual pretende corrobarar la subjetiva opinión, que desde el departamento de Pediatría del Hospital General Universitario de Elche tenemos, sobre que en Elche existen más niñas que presentan pubertad precoz y adelantada que en otras ciudades, como por ejemplo Valencia, que será la ciudad que nos servirá para realizar la comparación. Por otra parte, también nos gustaría corrobarar la sospecha de que el motivo de este aumento de pubertad precoz y adelantada en Elche es debido al contacto que mucha de nuestra población puede tener con la Industria del calzado, cuyos materiales podrían actuar como disruptores endocrinos que activen la pubertad en las niñas. Aprovecharíamos el estudio para conocer bajo qué factores de riesgo (como obesidad, genéticos) se encuentran las niñas de nuestras dos poblaciones así como conocer de una forma más precisa si la activación del proceso pubertal es más frecuente de forma central o periférica, ya que creemos que esta última está infravalorada y podría también indicarnos que la causa es de tipo exógeno, siendo las sustancias previamente citadas las encargadas de iniciar la pubertad.

Procedimientos

El objetivo de nuestro proyecto es conocer si su hija está comenzando la pubertad y cual podría ser el motivo de este inicio precoz o adelantado. Para ello necesitamos realizar la palpación del pecho, que nos indicará si se ha desarrollado la glándula mamaria. En aquellas niñas que por causa de una acumulación de grasa no sea posible distinguir la glándula, realizaremos una ecografía. Ambos procedimientos los realizaríamos en el colegio, en un lugar habilitado para que se haga de forma privada. Los dos son rápidos de prácticar e inocuos en cuanto a dolor y radiación se refieren. Las exploraciones se realizarían en dos ocasiones durante un año de margen durante los meses de enero y febrero. Se realizaran de forma privada, conservando la intimidad de la niña, en todas aquellas que sean residentes en Elche desde hace mínimo cuatro años.

Una vez realizada la exploración inicial, aquellas niñas que hayan presentado el inicio de desarrollo de la glándula, serán citadas en el hospital para la realización de otra ecografía, esta de la zona pélvica, que nos proporcione información sobre si las gónadas internas (útero y ovarios), están también iniciando el desarrollo pubertal. Aquellas niñas que se encuentren en un punto intermedio entre el estado prepubertal y pubertal, serán sometidas a un test llamado de GnRH, donde a través de una vía se realizará la infusión de la hormona central, para ver si existe respuesta o no de forma periférica. Este proceso nos permetirá diferenciar si la causa del inicio precoz o adelantado, es de tipo central o periférico.

Por otra parte, para poder conocer la etiología de la patología, necesitaremos que usted o aquella persona encargada de la hija, rellene un cuestionario, que contendrá los siguientes apartados: edad en que la madre tuvo su primera menstruación, la etnia de la participante, si tuvo o no bajo peso al nacer (menos de 2,500 gr), si es adoptada, su talla, peso y cálculo de masa corporal (que será medido por una enfermera en la misma consulta) y por último si la participante tiene contacto con la industria del calzado, que será positivo si alguna persona que viva en la misma casa, trabaja en cualquier momento de la semana en una fábrica de calzado, desde hace cuatro años, como mínimo.

Aquellas familias que así lo deseen entrarían en un grupo, donde se realizarían visitas continuadas para asesorar sobre el proceso de pubertad precoz y adelantada.

En la siguiente tabla se indica el calendario de las visitas y las pruebas a efectuar.

Visitas y determinaciones a efectuar

Medidas clínicas	**Inicial**	**12 meses**	**14 meses**	**16 meses**
Exploración: palpación pecho +/- ecogafía mamaria	X	X		
Ecografía pélvica +/- Test de GnRH			X	
Peso, talla, índice de masa corporal			X	
Cuestionario			X	
Seguimiento				X

Las exploraciones físicas, ecografía y test funcional de GnRH carecen de efectos secundarios conocidos, y se utilizan de manera rutinaria en el diagnóstico y control de pacientes con pubertad precoz y adelantada. La ecografía mamaria y pélvica se realiza en 5-10 minutos, es indolora y tampoco imparte irradiación. Por su parte, el test funcional de GnRH, consiste en la infusión de una sustancia llamada Luforan y la extracción de sangre basales y posteriormente a los 10, 20, 30, 60 y 90 minutos, la introducción de la aguja puede provocar cierta molestia y puede quedar cierto grado de hematoma, para la medición se necesita que los pacientes se mantengan tumbados hasta que se realice la última extracción.

Posibles beneficios
La participación de su hija en el estudio implica conocer si está iniciando el proceso de pubertad antes de lo que debería y descifrar cual podría ser el motivo de ello.
Esto constituye una fuerte herramiento de conocimiento sobre nuestra población femenina y bajo que factores pueden estar implicados en su salud.
Esto no solamente podría ser importante para este grupo, sino para toda la población, ya que los disruptores endocrinos descritos anteriormente también suponen un factor de riesgo para toda la población, puesto que también interactuando con otros sistemas del cuerpo humano: como el sistema cardiovascular, el cerebro, el pecho o la próstata.
Conocer toda esta información nos permetería poder hacer importantes programas de salud pública para la prevención y tratamiento.

Riesgos e inconvenientes
Los procedimientos realizados, como ya hemos comentado anteriormente no deberían producir ningún efecto indeseado para las pacientes, ya que no se han demostrado alguno hasta el momento.

El tiempo para conocer si existe o no inicio de desarrollo pubertal puede realizar un poco extenso, pero totalmente necesario para la buena realización del estudio y para poder encontrar resultados, por tanto instamos tanto a los participantes como a ustedes, padres y madres/tutores legales que sean pacientes con el proceso.
Durante el proceso, podrían surgir algunas dudas tanto en las participantes del estudio como en ustedes, padres y madres/tutores legales, por tanto invitamos a que manifiesten y comuniquen sus dudas a la investigadora principal (persona Dr. Irene Ripoll Murcia,tel 966 61 69 00. ext. 2810; móvil: 617684280).

Participación voluntaria
La participación de su hija en este ensayo clínico es voluntaria, por lo que, aunque inicialmente aceptara participar, usted podrá solicitar a los responsables del estudio, en cualquier momento y sin necesidad de especificar el motivo, la baja del estudio así como la eliminación de toda la información recogida, sin que esto repercuta en sus cuidados médicos.
Puede comentar la información recibida con su familia, con su médico o con quien considere oportuno para sentirse bien aconsejado. El médico del estudio le contestará a cualquier pregunta o duda que no haya quedado clara.

Compensación

Su participación en el estudio no le supondrá ningún gasto y le serán reintegrados los gastos extraordinarios (p. ejem. comidas y traslados) si usted lo solicita. Usted no tendrá que pagar por las pruebas del estudio.

Confidencialidad
Todos los datos de carácter personal e información recogida o generada en el estudio quedaran protegidos de acuerdo a la legislación vigente sobre protección de datos de carácter personal (Ley Orgánica 15/1999 del 13 de diciembre). Nadie, excepto su médico y el personal directamente relacionado con este estudio, podrá conocer su identidad. Únicamente las autoridades sanitarias podrán tener acceso a las secciones relevantes del estudio, si así lo solicitaran.