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RESEARCH

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Alterations of the hormones follitropin and lutropin in the blood of young mice dosed with lindane

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Abstract

Background The gonadotropins lutropin and follitropin stimulate steroid synthesis in the gonads during gonadal maturation by promoting oogenesis and spermatogenesis. Endocrine disruptors such as lindane (γHCH) can alter the reproductive stage so and could alter puberty by interfering with lutropin and follitropin signaling. γHCH was classified by World Health Organization as "moderately restricted", and in 2009 it is production and agricultural use was prohibited, under the Stockholm Convention, however, its use as a pharmaceutical treatment against lice and scabies is still allowed (and mainly, for use by the health sector). This study aimed to examine the effects of single-dose exposure to γHCH in young mice (*Mus musculus*) on lutropin and follitropin concentration, and to correlate gonadal maturity status and phenotypic characteristics.

Methods Young γHCH-treated mice (16 females and 16 males) received a single dose of 25, 10, 5, or 0 µg/ml γHCH per gram of body weight, ocular route. Once secondary sexual characteristics were observed, the gonads were dissected and examined using histological techniques and the ovarian follicles were classified as dependent and independent of gonadotropins; the testicles were classified as inactive, early and late spermatogenesis; and the blood was processed with the lutropin and follitropin ELISA kit.

Results The results indicate significant differences in the concentration of lutropin and follitropin between males and females (Fisher $p < 0.05$). The lutropin and follitropin levels showed a tendency to decrease in females, whereas, in males they tended to increase as the dose of γHCH increased. In females, it was observed that at the follitropin and lutropin decreased 42.3% and 83.7%, respectively at dose of 25 µg/ml; while in males, follitropin increased 51.9% at the dose of 25 µg/ml, and at the dose of 10 µg/ml lutropin increased by 242.5%. Contrary to that, gonadal maturity increased in females and decreased in males with increasing γHCH dose, disagreeing with the idea that gonadotropins coincide with the onset of puberty and gonadal maturity.

Conclusions The results allow us to infer that exposure to γHCH could promote non-monotonic responses; however, this does not seem to alter puberty in the doses and conditions of the present experiment.

Keywords Puberty, Early puberty, *Mus musculus*, Endocrine disrupting chemical, Gonadotropins

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Background

During the pubertal stage, preparatory hormonal signaling occurs (Kolby et al. 2017; Delli et al. 2023), which is susceptible to endocrine disrupting chemicals (EDCs) exposure capable of altering hormonal processes and compromising adult reproductive function (Dickerson et al. 2011; Marty et al. 2011; Ghosh et al. 2022). As early puberty signals, gonadotropin-releasing hormones (GnRH) promote the release of lutropin (LH) and follitropin (FSH), which incite the production of sex steroid hormones and stimulate sexual maturation (Abdelaziz 2023; Fanis et al. 2023). The pulsatile increase in LH and FSH indicates the characteristic biological signs of pubertal maturation (Léger and Carel 2016; Brambilla et al. 2023), as the advent of secondary sexual characteristics (SSC) (Chotipakornkul et al. 2023).

In mammals, LH and FSH are the main hormones transported in the blood (Brambilla et al. 2023); however, they act differently between males and females. In females, FSH binds to specific receptors on granulosa cells and induces the development and maturation of ovarian follicles (Björvang et al. 2022). In contrast, LH triggers ovulation by stimulating the maturation of the Graafian follicles and the development of the corpus luteum (Deligdisch-Schor and Mareş Miceli 2020; Zhang et al. 2022). In males, FSH stimulates the proliferation of Sertoli cells, maintains sperm quality, stimulates the synthesis and inhibition of anti-Müllerian hormone, and initiates spermatogenesis and testicular maturation (Abdelaziz 2023); while LH binds to Leydig cell receptors, increases adenosine monophosphate (AMP) and promotes testosterone secretion (Zhang et al. 2022; Abdelaziz 2023; Wang et al. 2023). Increased gonadotropin concentrations stimulate the late stages of spermatogenesis and oogenesis (Lefebvre et al. 2023; Zhao et al. 2023). Changes in the pattern of GnRH secretion produce an increase in the frequency and amplitude of LH and FSH pulsatility, which increases the secretion of steroids in the gonads, responsible for the development of SSC (Villanueva and de Roux 2016).

Although the relationship of EDCs to certain reproductive diseases has been documented (Ghosh et al. 2022), few studies have examined the direct impact of EDCs on fertility. Fewer investigations link the effect of EDCs at critical stages of development (Cabry et al. 2020), and very few are the works that consider the effects at low doses of EDC (Kim et al. 2018; Gan et al. 2023). This is known that altering the concentration of FSH, during the prepubertal period, accelerates puberty and positively affects testicular development in males, because spermatogenesis requires the direct action of FHS on the spermatogenic (Villanueva and de Roux 2016; Harstine et al. 2018; Vezzoli et al. 2023). EDCs such as γHCH (lindane; γ-hexachlorocyclohexane) can act on gonadotropin-releasing hormone (GnRH) and gonadotropins, promoting suppression of ovarian steroidogenesis, steroid hormones, and free cholesterol (Singh and Singh 1991; Björvang et al. 2022; Lambert and Bouvattier 2022).

γHCH is an organochlorine classified among persistent organic compounds considered an endocrine disruptor because it is neurotoxic and carcinogenic. In Mexico it is used to control ectoparasites in livestock and domestic animals; It is also used in agriculture as a seed treatment (Avalos Gómez and Ramírez Gutiérrez 2003). The continuous use of lindane promotes its presence in the environments, and due to its physical–chemical characteristics it could be transported atmospherically and enter ecosystems, and bioaccumulate and biomagnify through the food web (Kolani et al. 2017; Ali et al. 2021). The consumption of contaminated foods (seeds, water and meat) puts environmental and public health at risk, which includes humans (Requena-Mullor et al. 2021). Although γHCH can alter the reproductive stage, this is unknown how their affects the critical stages at the beginning of reproductive development, such as: the pubertal and prepubertal stages. Due to the importance of the pubertal stage, this is necessary to know the effect of γHCH an EDC capable of altering the reproductive establishment of both males and females (Ghosh et al. 2022), and provide evidence for the hypothesis that EDCs promote the onset of puberty, mainly when exposure occurs during the critical developmental stages of embryo implantation and prior to puberty (Tena-Sempere 2010). This study aimed to examine the effects of exposure to a single dose of γHCH at concentrations of 25, 10, 5 and 0 μg/mL in young mice (*Mus musculus*) on the concentration of LH and FSH, and to correlate the state of gonadal maturity and phenotypic characteristics.

Methods

Due to the similar characteristics in the reproductive system with humans, rodents are excellent experimental models to study the effects that EDCs promote on reproduction and puberty (Paris et al. 2016). In this investigation, 12 four-week-old mice (*Mus musculus*) were used as parents, they were confined in cages designed to group a male with three females. Three groups were formed and maintained at controlled temperatures of 27 ± 3 °C, and with alternating 14/10-h light/dark cycles. Food (Purina® NutriCubes) and water were available ad libitum. The handling of the animals was carried out following the ethical guidelines and principles of the National Research Council of the National Academies (2011), and the suggestions by Benavides and Guénet (2003). From the groups of parents, 32 offspring (16 females and 16 males) were obtained, which were used in the experiment

(Table 1). At 18 days of age (DOA), the mice were ocularly treated with a single dose of 1µL per gram of body weight, at concentrations of 25, 10, 5 and 0 (control) µg/mL of γHCH (Sigma-Aldrich®, 1000 µg/mL on methanol, part number: 40102) in sweet almond oil as vehicle. All mice were weaned at 21 DOA. Starting on DOA day 22, mice were weighed daily and the DOA at which SSCs appeared was monitored. On the day the SSCs appeared, the mice were anesthetized and euthanized according to Álvarez-Romero and Medellin (2005).

To measure the concentration of the LH and FSH hormones, a blood sample was obtained from the jugular vein using a BD Vacutainer® tube with EDTA, which was stored at −70 °C until further analysis in the laboratory. The blood sample (cell lysates) was centrifuged at 3500 rpm for 15 min, and the supernatant was placed in a microwell plate of the ELISA kit (BIOMATIK, Luteinizing hormone Cat.No. EKE61498; follicular stimulating hormone Cat.No. EKE61638) and the manufacturer´s protocols were followed. The calibration curve was worked together with the samples, at concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0 ng/mL for FSH (R2 = 0.98), and for LH of 30, 15, 7.5, 3.75, 1.88, 0.94, 0.47 and 0 ng/mL (R2 = 0.97).

Gonadal tissues were removed and placed in 10% formalin for 24 h. Subsequently, they were processed following the fixation protocols in the histokinet equipment (Microm STP120, Thermo-Fisher Scientific; Inc., Waltham, MA, USA), followed by paraffin embedding (Faga Lab; FAVELA PRO S.A. de C.V., Sin. Mex.) with the inclusion center (Histostar, Thermo-Fisher Scientific, Inc.). A microtome (Microm HM 340E, Thermo-Fisher Scientific, Inc.) was used to obtain 4 µm-thick sections of the tissues, that were mounted on slides pretreated with USP/NF gelatin (Fermont; Monterrey Chemical Products, NL, Mex.). Samples were stained with hematoxylin and eosin and observed under a Leica microscope (coupled with a camera) with 4x, 10x, 40×and 100×objectives (Leica ICC50; Leica Biosystems, Deer Park, IL, USA). The images of the histological samples of the ovaries and testes were analyzed visually. Ovarian follicles were classified according to Pedersen (1970) into two groups: (1) follicles with independent growth of gonadotropins (G.I.), formed by small follicles (consisting of germinal cells and primordial (Prd) cell cysts with oocytes smaller than 20 µm), and medium follicles (consisting of primary, secondary I and secondary II, with oocytes between 20 and < 300 µm); and, (2) gonadotropin-dependent growth follicles (G.D.), formed by late oogenesis with large follicles (consisting of antral follicles (Ant), preovulatory, Graafian follicle, ovulation, corpus luteum (CL) and atretic follicles, with oocytes > 300 µm). The testes were classified according to Bernard and Hall (1995), in three groups: (1) inactive spermatogenesis (I.S.), with elongation of tubules, spermatogonia, and Sertoli cells; (2) early spermatogenesis (E.S.), with seminiferous epithelium comprising spermatogonia and spermatocytes, but no spermatids; and 3) late spermatogenesis (L.S.), with spermiogenesis and sperm in the lumen of the seminiferous tubules.

Results were expressed as mean, minimum/maximum, and standard deviation. The differences with respect to the control were calculated. The statistical analysis was carried out with the Microsoft Excel® and Statistix 8.0® program; the Fisher test ($p < 0.05$) and the Pearson correlation test ($p < 0.05$) were used to correlate the concentration of the LH and FSH hormones with the stage of gonadal maturity and phenotypic characteristics (DOA of the SSC, weight and height).

Results

The DOA of the SSC, weights, height, gonadal maturity, and FSH and LH showed inversely between the sexes of the mice dosed with γHCH compared to the control (Table 2). In the females dosed with 25 µg/mL of γHCH, a 14.6% decrease was observed in DOA, 19.1% in weight, 3.3% in height, and 42.3% and 83.7% in the concentration of FSH and LH, respectively. In males, different responses

Table 1 Experimental design: selection of offspring per litter (by sex) from randomly arranged parents

Parents and litters 0 γHCH µg/mL (control)	**Female 1:Male 1** Breeding 1 Offspring 1♀:1♂	**Female 1:Male 2** Breeding 2 Offspring 1♀	**Female 2:Male 1** Breeding 3 Offspring 1♀:	**Female 3:Male 3** Breeding 4 Offspring 1♀:1♂
Treatments	**Female 3:Male 3** Breeding 5	**Female 6:Male 1** Breeding 6	**Female 5:Male 2** Breeding 7	Female 4:Male 3 Breeding 8
γHCH µg/mL	Offspring	Offspring	Offspring	Offspring
0 (control)	1♂	1♀	1♂	0
5	2♂	1♀:1♂	1♂	2♀
10	1♀	1♀:2♂	1♀:1♂	1♀:1♂
25	1♀	1♀:2♂	1♀:1♂	1♀:1♂

Table 2 Means of DOA at the advent of the SSC, weight, lengths, and concentration of FSH and LH of young mice of both sexes treated with γHCH

Dose µg/mL	Female					Male				
	DOA	**Weight**	**Height**	**FSH**	**LH**	**DOA**	**Weight**	**Height**	**FSH**	**LH**
Control (0 γHCH)	32.8[a]	16.7[a]	158.8[ab]	13.66	7.01	23.5	10.3	132[b]	10.84	1.62
5 γHCH	28.5[b] (↓*13.1%)*	14.8[a] (↓*11.3%)*	154.3[ab] (↓*2.8%)*	13.05 (↓*4.3%)*	3.10 (↓*55.7%)*	23.8 (↑*1.2%)*	11.9 ***(↑15.5%)***	140.5[a] ***(↑6.4%)***	15.96 (↑*47.2%)*	3.86 (↑*138.2%)*
10 γHCH	29.8[ab] (↓*9.1%)*	16.4[a] (↓*1.7%)*	160.8[a] (↑*1.2%)*	15.27 (↑*11.7%)*	6.70 (↓*4.4%)*	24.8 ***(↑5.5%)***	11.9 ***(↑15.5%)***	139.5[ab] (↑*5.6%)*	11.82 (↑*9.04%)*	5.55 ***(↑242.5%)***
25 γHCH	28[b] ***(↓14.6%)***	13.5[b] ***(↓19.1%)***	153.5[b] ***(↓3.3%)***	7.88 ***(↓42.3%)***	1.14 ***(↓83.7%)***	24.8 ***(↑5.5%)***	11.7 (↑*13.5%*)	137.5[ab] (↑*4.1%)*	16.47 ***(↑51.9%)***	3.54 (↑*118.5%)*

DOA = the days advent of the SSC. The arrows indicate the increase (↑) or decrease (↓) of the percentage of differentiation (in italics) relative to the control. The highest percentages according to variable are marked in bold. Superscript letters are different groups without significant statistical differences (Fisher's test $p < 0.05$)

were observed at doses of 5, 10, and 25 µg/mL of γHCH, with a 5.5% increase in DOA, 15.5% in weight, 6.4% in height, and 51.9% and 242.5% in FSH and LH concentration, respectively. In general, females recorded higher averages than males in DOA, weight, height, FSH and LH. In females, the dose of 10 µg/mL of γHCH, compared to the other doses, registered the highest mean data for DOA, weight, height, FSH and LH. In males, the dose of 5 µg/mL of γHCH, compared to the other doses, registered the highest means. Different groups are observed among the DOA, weights, and heights per dose of γHCH without significant statistical difference (Fisher's test $p = 0.05$).

A strong positive correlation was observed between FSH and LH and the size of males exposed to a dose of 5 µg/mL of γHCH, and a strong positive correlation between LH and the size of females exposed to a dose of 25 µg/mL of γHCH, with statistically significant differences (Pearson, $p < 0.05$). In the control, a strong negative correlation was observed between heights and weights and FSH of females (Table 3), with a statistically significant difference (Pearson, $p < 0.05$). In females, the most significant differences, compared to the control group, were registered in the reductions of FSH and LH concentrations for DOA with 159% and 561%, respectively, when γHCH was used at 5 µg/mL and in FSH and LH with weight and height with 152% and 176%, and 63% and 200% respectively, in the dose of 25 µg/mL of γHCH. For LH of females, an increase in differentiation was observed, at a dose of 10 µg/mL of γHCH in DOA, compared to the control, 130% for DOA, 21% in weight and 181% in height; differentiation increases in FSH of females were not observed. In males, the highest percentage of differentiation with respect to the control was registered in FSH by DOA with a 134% increase in the 25 µg/mL dose of γHCH. In contrast, weight and height experienced a 311% and 400% decrease, respectively, when exposed to a 5 µg/mL dose of γHCH; LH in male led to a 305% increase in DOA, while weight and height experienced a 160% and 144% decrease, respectively, at the 10 µg/mL dose of γHCH.

Gonadal maturity was compared with gonadotropin concentrations at different doses of γHCH (Table 4). In females, FSH and LH decreased, while G.I. decreased and G.D. increased with increasing γHCH dose. Different groups are observed without significant statistical differences between G.I. and G.D. with FSH and LH (Fisher´s test $p < 0.05$). In males, the concentration of FSH and LH showed an increase, while I.S. increased, and E.S. and L.S. decreased as the γHCH dose was increased. No different groups or significant statistical differences (Fisher's test $p < 0.05$) were observed between I.S., E.S., and L.S. with FSH and LH.

Correlation between FSH and LH was observed in G.I. and G.D. in females, as well as in I.S., E.S., and L.S. in males, in all young mice exposed to γHCH (Table 5). The strong positive correlation (0.93) between FSH and L.A.S. of the control group (Pearson test $p < 0.05$) could reflect the normal behavior of gonadotropin on gonadal maturity. In the control, a correlation was observed between the concentration of FSH and G.D., in addition, this was observed that this decreased with the doses of 5 and 10 µg/mL of γHCH, while an increase of 80% was detected with the dose of 25 µg/mL of γHCH. The correlation of FSH with G.I. showed a progressive decrease with increasing γHCH dose. Unlike the control, FSH with I.S. decreased, while a progressive increase was observed with E.S. and L.S. as the γHCH dose increased. For LH, when compared with the control, in G.D. and G.I. the correlation seems to decrease with exposure to γHCH except for the 10 µg/mL dose of γHCH in G.D. and 25 µg/mL of γHCH in G.I. where the percentage of

Table 3 Pearson's correlation coefficient between gonadotropins and phenotypic characteristics of γHCH-treated juvenile mice and percentages of differentiation relative to control

Dose µg/mL	FSH						LH					
	DOA	Female Weight	Height	DOA	Male Weight	Height	DOA	Female Weight	Height	DOA	Male Weight	Height
Control (0 γHCH)	-0.77	-0.92	-0.94	0.29	-0.27	-0.33	0.13	0.19	0.32	0.20	0.73	0.77
5 γHCH	0.46 (↓***159%***)	-0.23 (↓*75%*)	0.49 (↓*47%*)	0.47 (↑*62%*)	0.57 (↓***311%***)	**0.99** (↓***400%***)	-0.60 (↓***561%***)	0.22 (↑*15%*)	0.57 (↑*78%*)	0.53 (↑*165%*)	0.55 (↓*24%*)	**0.99** (↑*28%*)
10 γHCH	-0.62 (↓*19%*)	-0.15 (↓*83%*)	-0.62 (↓*34%*)	0.46 (↑*58%*)	0.21 (↓*177%*)	-0.42 (↑*27%*)	0.30 (↑*130%*)	0.23 (↑*21%*)	0.90 (↑*181%*)	0.81 (↑***305%***)	-0.44 (↓***160%***)	-0.34 (↓***144%***)
25 γHCH	-0.05 (↓*93%*)	0.48 (↓***152%***)	0.72 (↓***176%***)	0.68 (↑***134%***)	-0.86 (↑*218%*)	-0.82 (↑*148%*)	-0.53 (↓*507%*)	0.07 (↓***63%***)	**0.96** (↑***200%***)	0.28 (↑*40%*)	0.55 (↓*24%*)	0.73 (↓*5%*)

DOA = the days at advent of the SSC. Numbers in red indicate significant statistical differences (Pearson, $p < 0.05$). The arrows indicate the increase (↑) or decrease (↓) of the percentage of differentiation (in italics) relative to the control. The highest percentages according to variable are marked in bold

Table 4 Percentage of follicles and seminiferous tubules by stages of young mice treated with γHCH

Dose µg/mL	Female		Male		
	G.I	G.D	I.S	E.S	L.S
Control (0 γHCH)	80.3[a]	19.7[b]	65.8	23.0	11.1
5 γHCH	76.9[ab] *(↓4.2%)*	23.1[ab] *(↑17.2%)*	97.4 *(↑48.02%)*	2.3 *(↓90%)*	0.17 *(↓98.4%)*
10 γHCH	69.8[b] *(↓13.07%)*	30.2[a] *(↑53.2%)*	99.7 ***(↑51.5%)***	0.3 ***(↓98.6%)***	0 ***(↓100%)***
25 γHCH	66.5[b] ***(↓17.1%)***	33.5[a] ***(↑70.05%)***	98.8 *(↑50.1%)*	1.1 *(↓95.2%)*	0 ***(↓100%)***

G.I. = independent follicles. G.D. = dependent follicles. E.S. = inactive spermatogenesis. I.S. = early spermatogenesis. L.S. = late spermatogenesis. Superscript letters indicate different groups without significant statistical differences (Fisher's test $p < 0.05$). The arrows indicate the increase (↑) or decrease (↓) of the percentage of differentiation (in italics) relative to the control. The highest percentages according to variable are marked in bold. Superscript letters are different groups without significant statistical differences (Fisher's test $p < 0.05$)

Table 5 Pearson's correlation coefficient between gonadotropins and stages G.D., G.I., I.S., E.S. and L.S. of young mice treated with γHCH and percentages of differentiation in relation to the control

Dose µg/mL	FSH					LH				
	Female		Male			Female		Male		
	G.I.	G.D.	I. S.	E. S.	L. S.	G.I.	G.D.	I. S.	E. S.	L. S.
Control (0 γHCH)	0.86	-0.35	-0.55	-0.19	**0.93**	-0.35	0.44	0.74	-0.29	-0.77
5 γHCH	0.48 *(↓44%)*	-0.12 *(↓65%)*	0.32 *(↓158%)*	-0.32 *(↑68%)*	-0.26 ***(↓127%)***	-0.19 *(↓45%)*	-0.44 ***(↓200%)***	0.26 ***(↓64%)***	-0.26 *(↓10%)*	-0.20 ***(↓74%)***
10 γHCH	-0.23 *(↓126%)*	-0.04 ***(↓88%)***	0.34 *(↓161%)*	-0.34 *(↑78%)*	nd	0.81 ***(↓331%)***	0.87 *(↑97%)*	0.74 *(0%)*	-0.74 ***(↑155%)***	nd
25 γHCH	-0.81 ***(↓194%)***	-0.63 *(↑80%)*	0.54 ***(↓198%)***	-0.54 ***(↑184%)***	nd	-0.43 *(↑22%)*	-0.27 *(↓161%)*	0.49 *(↓33%)*	-0.49 *(↑69%)*	nd

G.D. = gonadotropin – dependent oocytes. G.I. = oocytes independent of gonadotropins. E.S. = inactive spermatogenesis. I.S. = early spermatogenesis. L.S. It is late spermatogenesis. Numbers in red indicate significant statistical differences (Pearson, $p < 0.05$). The arrows indicate the increase (↑) or decrease (↓) of the percentage of differentiation (in italics) relative to the control. nd = data not available. The highest percentages according variable are marked in bold

differentiation increased. When compared to the control, the percentage of differentiation between LH and I.S., E.S. and L.S. appears to decrease, increase, and decrease, respectively, except for concentrations of 10 µg/mL of γHCH in I.S. and 5 µg/mL of γHCH in E.S.

Discussion

Results indicate that there is no evidence to suggest that γHCH promotes changes at the onset of puberty. However, this seems to promote possible non-monotonic responses in LH and FSH, as well as subtle changes in gonadal maturity and phenotypic characteristics (CSS DOA, weight and height). The concentrations of FSH and LH showed a decreasing trend in females and an increasing trend in males, as the dose of γHCH increases, also a higher proportion of FSH was detected compared to LH. However, the 10 µg/mL dose of γHCH showed a non-monotonic response when females presented the highest concentrations of FSH and LH, whereas, in males, FSH was lower and LH higher among the groups dosed with γHCH. Cooper et al. (1989) dosed female rats at concentrations of 0, 5, 10, 20, and 40 mg/kg of γHCH and observed that the FSH concentration was higher, whereas the LH concentration was reduced, compared to the control group, similarly to the results of the present study. In addition, they observed significant delays in vaginal opening at 10 and 40 mg/kg of γHCH, whereas, in our study, at a dose of 10 µg/mL of γHCH an increase in DOA was observed in females compared to the dosed groups. Non-monotonic dose–response results for EDCs continue to challenge basic assumptions of toxicology and risk assessment (Lefebvre et al. 2021, 2022).The latter is mainly due to the mechanisms of action of low-dose effects, and the relevance of critical periods of exposure (Ghosh et al. 2022) makes it difficult to predict the consequences of exposure. This finding contributes to the hypothesis that low doses show non-monotonic dose–response relationships, in contrast to those observed at high doses (Marty et al. 2011; Vandenberg et al. 2012; Lefebvre et al. 2021), because γHCH is recognized as a xenoestrogen (Paris et al. 2016) and, as stated by Xuereb et al. (2011) and Ghosh et al. (2022), EDCs classified as xenoestrogens show non-monotonic dose-responses. The latter agrees with Marty et al. (2011) who propose that

low doses promote subtle biological changes that occur within exposure ranges at lower doses than those conventionally used in reproductive and developmental toxicity tests. These subtle biochemical effects, occurring at low doses, suggest the initial phase of toxicity without causing potential effects on the health of organisms and, probably, indicate of adaptive compensatory mechanisms in organisms (Dikshith et al. 1989), or an evolutionary adaptive process, resulting from mitohormesis or xenohormesis/hormesis (Kim et al. 2018; Erofeeva 2023).

In this work, the females dosed at 25 µg/mL of γHCH, compared with the control, had lower concentrations of FSH and LH with negative correlations and with a reduction in DOA, in which the SSC, weight and height were presented, in addition of minor G.I. and greater G.D., which reflects greater gonadal maturity unrelated to gonadotropins. The results of this study appear to be inconsistent with what is indicated by Pawlina and Ross (2015), who point out that the maturation of the sexual organs, both internal and external, during puberty is mediated by estrogens (steroids) promoted by gonadotropins, which foster the maturation of follicles (Brambilla et al. 2023; Gea et al. 2023). According to Villanueva and de Roux (2016) and Dickerson et al. (2011) the main indicator of puberty is given by the increase in gonadotropin secretion, just before the onset of SSCs, which coincides with the onset of puberty and gonadal maturity (Micangeli et al. 2023). Wordinger and Highman (1984) indicate that follicular growth is correlated with gonadotropin hormones; FHS induces follicle growth and Ant formation in secondary follicles 2 (follicles present in G.I.); whereas LH acts on the G.D. ranging from Ant follicles to ovulation (including CL and atresia) (Beck et al. 2024). In the G.I. of controls a strong positive correlation with FSH and weak a negative on with LH were observed, but, in the 25 µg/mL γHCH treatment, the correlation was strongly negative with FSH and weakly negative with LH, which could indicate that the follicles in G.I. are not influenced by FSH due to the presence of γHCH. For the follicles in G.D., it was observed that the controls had a weak negative correlation with FSH and a medium positive correlation with LH, while the follicles treated with 25 µg/mL of γHCH presented a strong negative correlation with FSH and a weak negative correlation with LH, suggesting that γHCH interferes with LH influencing follicle maturation. In both cases, our results seem to contrast to the proposal by Wordinger and Highman (1984) and Dickerson et al. (2011) because statistically there is no correlation between the stage of maturity and gonadotropins. According to Duran Reyes et al. (1997) and Xu et al. (2024), follicles present a follicular microenvironment known as follicular fluid-of varied biochemistry-(Lefebvre et al. 2023) that regulates oocyte development and the different stages of follicular maturity. In fact, these authors detected that secondary follicles 1 and 2 have high concentrations of estradiol, whereas high concentrations of progesterone were observed in the Ant and Graff's follicles. In addition to having steroids, this follicular fluid is rich in lipids and proteins, which according to Yang et al. (2012), can be detrimental to oocytes. This is mainly due to the fact that contaminants move from one place to another, depending on the mobilization of lipids induced by the hormonal system (Lefebvre et al. 2022), and by the metabolic activity that demands lipids, such as the development of follicles. Likewise, Al-Hussaini et al. (2018) point out that the presence of EDC in the follicular fluid may negatively affect embryological results, suggesting that γHCH is accumulating in the follicular fluid due to different lipid contents, disrupting the follicles.

In the present study, males dosed at 5 µg/mL of γHCH presented significant statistical differences in the correlations with FSH, LH, and with increased weight and height, compared to the control group, with a positive effect of the gonadotropins on I.S. and a negative on E.S. and L.S. In addition to this, at the 10 µg/mL dose of γHCH, the highest concentration of LH and FSH, greater weight and I.S. were recorded with lower values in E.S. and L.S., reflecting a greater immaturity in the organisms exposed to γHCH. Pawlina and Ross (2015) indicates that LH stimulates Leydig cells to release testosterone and initiate spermatogenesis and testicular descent; while FSH stimulates Sertoli cells and promotes spermiogenesis (Ghosh et al. 2022; Stafford 2023); during the prepubertal period Sertoli cells proliferate until the blood barrier of the testis is established before puberty (Harstine et al. 2018). The administration of γHCH caused a faster testicular descent compared to the control group, however, a greater amount of immature seminiferous tubules, developed to seminiferous cords, were recorded in I.S. This is known that LH promotes SSC and the maturity of the seminiferous tubules (Chotipakornkul et al. 2023), however, in this study, no correlation was observed with both hormones in the L.S maturity stage. This finding is consistent with that reported by Wordinger and Highman (1984) regarding testicular descent, however, in the current research no stimulation of spermiogenesis or spermatogenesis was observed. Although, Dickerson et al. (2011) propose that delayed puberty from EDC exposure may be due to an imbalance of the androgen: estrogen system and their receptors (Colvin et al. 2022; Lambert and Bouvattier 2022), EDC exposure has been shown to affect the number of sperm in the seminiferous tubules (Durand et al. 2020). It is known that lipid droplets are found around Leydig cells (Chung et al. 2020), which, in addition to steroids present in the tubules, could be the

anchoring site of γHCH, accumulating in these types of lipids and altering normal metabolic function. Based on the contrasting response of gonadal maturity and FSH and LH between females and males, disagreement with the general idea that EDCs equally affect females and males at critical periods of sexual differentiation is evident, with effect on gonadal function and development.

Conclusions

The EDC exposure affects gonadal maturity and gonadotropins but acts inversely between the sexes. Exposure to γHCH does not seem to alter puberty at the doses and conditions of the present experiment, however, this promotes subtle changes in gonadal maturity, in the concentration of FSH and LH, and in the DOA, in which CSS with possible non-monotonic responses are presented. this is suggested that γHCH possesses lipid selectivity, thus, further studies are required to identify γHCH anchoring sites.

Abbreviations

Lindane (γHCH)	γ-Hexachlorocyclohexane
EDCs	Endocrine disrupting chemicals
GnRH	Gonadotropin-releasing hormones
LH	Lutropin
FSH	Follitropin
SSC	Secondary sexual characteristics
DOA	Days of age
G.I.	Follicles with independent growth of gonadotropins
G.D.	Gonadotropin-dependent growth follicles
Ant	Antral follicles
CL	Corpus luteum
I.S.	Inactive spermatogenesis
E.S.	Early spermatogenesis
L.S.	Late spermatogenesis

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection by EPG, PYLM, FAFF, OLC, VMSS, and analyses were performed by EPG, MNHM; JDSC, CLBT, IEV, HAGO, JFAP, and DERB. The first draft of the manuscript was written by EPG and all authors commented on previous versions. All authors read and approved the final manuscript. This work is part of the doctoral thesis of EPG. The authors agree to participate in this manuscript and give their consent to publish it.

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Availability of data and materials

The datasets generated and/or analyzed during the current study, and included in this published article are available in the Ernestina Pérez-González repository, in Dropbox. https://www.dropbox.com/scl/fo/7ercdmcnl3kjq1hrb642m/h?rlkey=gj3zfee82qb8309274041shv3&dl=0.

Declarations

Ethics approval and consent to participate

The handling of the animals was carried out following the ethical guidelines and principles of the National Research Council of the National Academies (2011), and the suggestions by Benavides and Guénet (2003).

Consent for publication

'Not applicable' for that section.

Competing interests

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