ORIGINAL RESEARCH ARTICLE

Drug Safety 2008; 31 (6): 537-543
0114-5916/08/0006-0537/$48.00/0

Risk of Hypospadias in Newborn Infants Exposed to Valproic Acid During the First Trimester of Pregnancy

A Case-Control Study in Spain

Elvira Rodríguez-Pinilla,[1,2] *Consuelo Mejías*,[1] *David Prieto-Merino*,[3] *Paloma Fernández*[1] and *María L. Martínez-Frías*,[1,2,4] *on behalf of the ECEMC Working Group*

1 Spanish Collaborative Study of Congenital Malformations (ECEMC), Research Center on Congenital Anomalies (CIAC), Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Madrid, Spain
2 CIBER de Enfermedades Raras (CIBERER), Madrid, Spain
3 Medical Statistics Unit, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK
4 Department of Pharmacology, Faculty of Medicine, Universidad Complutense, Madrid, Spain

Abstract

Background: Hypospadias is one of the most frequently occurring genital anomalies described in infants prenatally exposed to valproic acid (VA). However, to our knowledge, only one publication has studied a potential causal relationship between VA and hypospadias, only estimating the unadjusted global risk. Here we present the results of a multivariate case-control study aimed at analysing and quantifying the specific risk of hypospadias in newborn infants exposed to VA during the first trimester of pregnancy.

Methods: The data analysed here were derived from the Spanish Collaborative Study of Congenital Malformations (ECEMC), an ongoing, hospital-based, case-control study and surveillance system in which collaborating paediatricians identify case and control infants. The paediatricians collect the same data for both case and control infants, blinded to information on any prenatal exposure. The information includes 312 items related to many prenatal exposures, including drug exposure, reproductive and family history, and other characteristics. The sample analysed included 2393 infants with hypospadias and 12 465 male controls.

Results: The results showed that the unadjusted risk of hypospadias in infants prenatally exposed to VA was 5.23 (95% CI 2.31, 11.86; $p < 0.00001$). Once adjusted for 13 potential confounding factors using conditional logistic regression analyses, the value of the risk was of a similar magnitude (odds ratio = 5.71; 95% CI 1.78, 18.36; $p = 0.003$). In addition, the frequency of hypospadias in the study population was approximately 1.8/1000 births. This allowed us to calculate the specific risk for an infant with hypospadias to be born to an exposed mother, which was 1 child in 97 births to mothers using VA during the first trimester of pregnancy. We consider this information much more useful for risk assessment than the risk value itself.

Conclusions: An alteration of placental gonadotrophic stimulation caused by changes in gonadotropin-releasing hormone release produced by the effects of VA on GABA is a possible pathogenic mechanism. Our results support the relationship between prenatal exposure to VA and hypospadias.

Background

Valproic acid (VA) is effective in inhibiting absence seizures as well as partial and generalized tonic-clonic seizures in humans. Currently, VA is also used in the treatment of bipolar disorder and other affective disorders. It is well known that prenatal exposure to VA increases the risk of neural tube and a number of other defects in newborn infants,[1-4] constituting the so-called 'fetal valproate syndrome'. This syndrome is characterized by features including facial dysmorphism, prominent metopic ridge, outer orbital ridge deficiency, bifrontal narrowing, cardiac defects, radial limb deficiencies, genital anomalies and developmental delay.[5-10] Among the genital anomalies observed in infants prenatally exposed to VA, hypospadias, which presents as a malposition of the urethral opening on the underside of the penis, is one of the most frequently described.[5,11-15] However, to the best of our knowledge only one study has analysed the potential relationship between VA exposure and hypospadias, only estimating the unadjusted global risk.[16]

Here we present the results of a multivariate case-control study aimed at analysing and quantifying the specific risk of hypospadias in newborn infants exposed to VA during the first trimester of pregnancy.

Materials and Methods

We used data from the Spanish Collaborative Study of Congenital Malformations (ECEMC) network. The ECEMC is a hospital-based case-control study and surveillance system, with a methodology aimed at investigating the characteristics of congenital defects, their clusters and causes. This network is made up of two groups; the data collecting (peripheral) group and the coordinating group.[17]

The peripheral group includes physicians in over 80 collaborating hospitals throughout Spain who, through their interest in congenital defects, collaborate with the ECEMC programme, following its common and strict methodology. The collaborating paediatricians are trained to assess major and minor anomalies that are described in a common operational manual. They examine the newborn infants just after birth and within the first 3 days of life, to identify the case and control newborns. Once these infants have been identified, described clinically and their data collected, the same paediatricians identify and interview the mothers of the case and control infants (also during the first 3 days after delivery) using defined protocols, gathering the same information (up to 312 different datapoints) from both groups of mothers. When the paediatricians select the case and control newborn infants, they are blinded to the different maternal and family data that they are going to collect. This information includes reproductive and family history, obstetrics data, maternal and paternal occupation, acute and chronic maternal diseases and any exposures during pregnancy such as drugs, alcohol and tobacco. All the information on case and control infants is sent to the coordinating group on a monthly basis. In Spain, 100% of the population is covered by the public health system and therefore, as 95% of the collaborating hospitals belong to the public health system, the ECEMC samples are representative of the Spanish population.

The coordinating group, located in Madrid, is composed of experts in congenital defects epidemiology, clinical teratology, dysmorphology, clinical genetics and cytogenetics. They code the congenital anomalies, perform the cytogenetic studies, including fluorescent *in situ* hybridization (FISH) techniques, and, in collaboration with the participating paediatricians in the peripheral group, they establish the diagnosis of the cases and perform the epidemiological analyses.

Case Definition

Once a paediatrician has identified a newborn infant with birth defects, this individual is selected as a case and all the anomalies detected (major and/

or minor) are described in detail in the ECEMC common protocol. In most instances, photographs, blood samples to enable high-resolution karyotyping and FISH techniques, imaging studies, pathology reports and the results of other studies are also gathered, sent to the coordinating group, stored and made available for review.

Control Definition

For each case, the next infant without birth defects of the same sex born in the same hospital is selected as a control subject. Thus, the controls are selected from the same population as the cases and are representative of those who, had they developed malformations, would have been selected as cases.

Drug Information, Coding and Exposure

In the ECEMC protocol, apart from an open-ended question on drug consumption in general, there are specific questions relating to drugs in 13 therapeutic groups, including over-the-counter medications. The information collected for each type of drug includes the brand name, daily doses, gestational age when administered and the duration of the treatment. Among these therapeutic groups, there is one that deals with anticonvulsant drugs. In addition, there are questions on maternal epilepsy and other chronic maternal diseases.

As stated previously, the ECEMC system allows the study of cases in relation to their clinical presentation, regardless of whether the underlying cause of the congenital defect is known or unknown.[17]

Study Samples of Case and Control Infants

Between January 1986 and December 2004, the ECEMC surveyed a total of 1 654 008 live births. Among them, 23 986 were identified as having major or minor congenital anomalies, and 22 860 infants without congenital anomalies were selected as controls. Of the total of infants with congenital anomalies, 2502 had hypospadias. For the present study, we considered only male infant controls (of the total of 22 986 controls, 12 817 were male), and we excluded those patients with hypospadias known to be caused by an underlying genetic or chromosomal syndrome and their respective controls, as well as those patients and controls whose mother did not answer the questions on epileptic drug use. Thus, the total sample analysed in this study included 2393 infants with hypospadias and 12 465 male controls without birth defects.

Male infants were considered to have been exposed if their mothers were treated with VA during the first trimester of pregnancy. Cases and control infants whose mothers did not use VA during the first trimester of pregnancy were considered as non-exposed. The clinical indication for the treatment was epilepsy in all exposed mothers.

Statistical Analyses

To calculate the unadjusted global risk, we used 2×2 tables of case and control infants to estimate the odds ratio (OR) and 95% CI and conducted Fisher's exact test when necessary. To perform the stratified analysis, the Mantel-Haenszel (M-H) procedure was used. To control for potential confounders, a conditional logistic regression analysis (Stata/SE 8.0, Stata Corp., Texas, US) was applied, using male control infants from the total sample of controls who could be matched to cases by the design variables (i.e. year and region of birth). The selected potential confounding factors were maternal age, maternal level of education, maternal epilepsy, other chronic maternal diseases, maternal ethnic group, first degree relatives with congenital malformations, first degree relatives with hypospadias, fever, smoking, alcohol consumption and maternal intake of vitamins, sexual hormones and anticonvulsant drugs (other than VA) during the first trimester of pregnancy. p-Values for the logistic regression analysis were calculated using the likelihood ratio test.

Results

The results of the current analysis show that prenatal exposure to VA increases the risk of hypospadias in the fetus, with a global and unadjusted risk value of 5.23 (95% CI 2.31, 11.86; $p < 0.00001$, table I). The same analysis was carried out stratifying the treatment of VA into monotherapy and polytherapy with other antiepileptic drugs (table II). In both strata, there were different values for the risk of developing hypospadias, suggesting a possible interaction. However, as the test of homogeneity for the M-H strata was not statistically significant (p =

Table I. Unadjusted risk of hypospadias after prenatal exposure to valproic acid during the first trimester of pregnancy

Group	Exposed	Unexposed	Total	Proportion of exposed
Cases	14	2 379	2 393	0.0059[a]
Controls	14	12 451	12 465	0.0011

a Odds ratio = 5.23 (95% CI 2.31, 11.86) p < 0.00001.

0.54), we calculated the combined OR (M-H), which gave a value of 3.52 (95% CI 1.55, 8.02; p = 0.0023).

Analysis of selected variables was carried out to identify those that could confound the unadjusted results. Table III shows 13 separate bivariate analyses between each factor and the main outcome. It was demonstrated that epilepsy, other chronic maternal diseases, fever during the first trimester, smoking during the first trimester, having first degree relatives with congenital defects, having first degree relatives with hypospadias and intake of antiepileptic drugs other than VA may all be confounding factors for the current analysis. Thus, a regression analysis was carried out to control for the potential effect of all those variables (table IV), and the results indicate that maternal therapy with VA increases the risk of having a child with hypospadias 5.71-fold (95% CI 1.78, 18.36; p = 0.003), independent of the effect of the included factors.

Discussion

Hypospadias is one of the most frequently occurring congenital defects in humans. The prevalence of hypospadias at birth (all types included) ranges from 4.75 per 10 000 births in some regions of China, to 37.63 per 10 000 in Israel.[17] The frequency in Spain during the period 1980–1985 (before the law was passed permitting voluntary termination of pregnancy due to fetal defects) was 18.10 per 10 000 newborn infants (35.69 per 10 000 newborn male infants), and along with congenital cardiac defects it is the most frequent congenital abnormality.[18] Therefore, one would expect this anomaly to be one of the most frequently observed in males among any group of individuals, including infants born to epileptic mothers, meaning the fact that hypospadias have frequently been described in infants exposed to VA does not necessarily imply that the drug increases the risk of hypospadias. To the best of our knowledge, the only epidemiological study published to date observed a global risk of hypospadias (OR 2.59, 95% CI 1.4, 4.7),[16] but there are particular characteristics of the study that make it difficult to interpret their results. First, it was based on a series of infants with malformations born to mothers with a positive history of first trimester drug-exposure. That is, all of the infants studied had malformations (both cases and controls), and all were exposed to drugs prenatally. This design complicates the interpretation of the results obtained because the OR derived from such a sample does not represent the risk of the exposure causing the defect.[19] Second, the results of the study were unadjusted for confounders. Thus, we considered it necessary to confirm this association by using a more appropriate methodology, and controlling for certain confounding factors.

The strength of using the ECEMC programme to study the teratogenic effects of drugs during pregnancy not only lies in the large and unbiased series of consecutive newborn infants that are studied, but

Table II. Stratified analysis according to the exposure to other antiepileptic drugs

Exposure	Exposed to VA	Unexposed to VA	OR (95% CI)	p-Value
Monotherapy				
Cases	6	2 369	2.86 (0.87, 8.45)	p = 0.04
Controls	11	12 432		
Polytherapy				
Cases	8	10	5.07 (0.91, 34.84)	p = 0.03
Controls	3	19		

OR = odds ratio; **VA** = valproic acid.

Table III. Relationship between the selected potential confounding factors and the risk of hypospadias in the newborn. Each row is a separate bivariate logistic regression between the factor and the outcome

Factors	Odds ratio (95% CI)	p-Value
Maternal age	1.00 (0.99, 1.01)	0.799
Maternal educational level	0.97 (0.91, 1.03)	0.312
Epilepsy	2.49 (1.57, 3.94)	<0.0001[a]
Other chronic maternal diseases	1.17 (1.03, 1.34)	0.018[a]
Fever during first trimester	1.28 (1.01, 1.61)	0.037[a]
Smoking during first trimester	0.89 (0.80, 0.98)	0.021[a]
Alcohol consumption during first trimester	1.04 (0.88, 1.22)	0.637
Ethnic group	0.95 (0.88, 1.01)	0.116
Relative with congenital malformations	4.41 (3.74, 5.20)	<0.0001[a]
Relative with hypospadias	48.52 (30.48, 77.24)	<0.0001[a]
Vitamins during first trimester	1.06 (0.97, 1.16)	0.198
Sexual during hormones first trimester	1.25 (0.94, 1.66)	0.122
Other antiepileptic drugs than VA	4.28 (2.30, 8.00)	<0.0001[a]

a These variables were included in the multivariate model (see table IV).

also in the way that several methodological biases are controlled or minimized.[20] Firstly, selection bias is unlikely to be introduced since the paediatricians select all consecutive newborns with any type of birth defects as cases without having any prior hypothesis or interest in a particular defect. The collection of data is not focused towards any particular study, but is a permanent and systematized registry to gather data independent of aetiology or causality. In addition, as the same paediatrician interviews the mothers of both the case and control infants, any bias that might be introduced in the interview will be the same for both case and control infants and thus, will not affect the results. Moreover, in the protocols of the ECEMC there are specific questions for the type of drugs belonging to each one of the 13 main therapeutic groups, including over-the-counter medications. The maternal interview is performed during the first 3 days after birth, when mothers who have just delivered an infant with congenital defects are not psychologically predisposed to answer questions on their recent pregnancy. The opposite occurs with mothers having delivered healthy infants (controls) during the same period, who collaborate actively and accept the interview. Consequently, the control mothers may respond more accurately than case mothers and thus, any potential bias would favour the null hypothesis.[21]

The mechanism by which VA may induce hypospadias in humans remains unknown. Experimental studies in rats have shown that the ano-genital distance was not reduced in male offspring exposed to VA *in utero*.[22] This contrasts with the classic effects of the antiandrogenic drugs such as flutamide, cyproterone or finasteride, leading some authors to consider that VA may not cause hypospadias in humans through its antiandrogenic effects.[22] How-

Table IV. Results of the conditional logistic regression analysis for having a child with hypospadias after prenatal exposure to valproic acid and other factors during the first trimester of pregnancy

Factors	Odds ratio (95% CI)	p-Value
Valproic acid during first trimester	5.71 (1.78, 18.36)	0.003
Epilepsy	0.62 (0.21, 1.84)	0.390
Other chronic maternal diseases	1.08 (0.93, 1.25)	0.290
Fever during first trimester	1.35 (1.05, 1.73)	0.021
Smoking during first trimester	0.86 (0.77, 0.96)	0.009
Relative with congenital malformations	1.71 (1.34, 2.19)	<0.0001
Relative with hypospadias	28.33 (16.41, 48.90)	<0.0001
Other antiepileptic drugs during first trimester	4.29 (1.35, 13.66)	0.014

ever, a possible pathogenic mechanism implicated in the association between VA and hypospadias could be the effect of VA on gonadotrophic stimulation, along with the concomitant effect on androgen production by embryonic testes. The embryonic production of testosterone is mediated by placental rather than hypophyseal gonadotrophin. It is well known that VA produces an increase in the levels of the neurotransmitter gamma aminobutyric acid (GABA). This neurotransmitter could stimulate the production of placental gonadotropin-releasing hormone (GnRH) and this hormone may control the local biosynthesis and secretion of human chorionic gonadotropin (hCG).[22] Moreover, GABA can also directly stimulate hCG secretion and hCG biosynthesis in human first trimester placenta tissue *in vitro*.[23] Keeping this in mind, it seems logical that the treatment with GABAergic drugs such as VA would provoke an increase in GnRH as well as hCG and fetal androgens. However, it is suspected that a decrease in androgen levels is linked with hypospadias, rather than an increase. In this sense it is important to note that while administration of a GnRH agonist increases gonadotropin (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]) concentrations in plasma, constant administration of GnRH desensitizes gonadotropins leading to a suppression of LH and FSH secretion, resulting in a decrease in gonadal steroid secretion. In fact, it has been observed that long-term treatment of males with GnRH agonist analogues leads to impotence, decreased libido and reversible oligospermia, due to a concomitant fall in serum testosterone concentration.[24] All these issues are highly suggestive, but it remains unclear whether long-term treatment with VA during gestation may produce this negative feedback loop with the consequent desensitization of hCG and decrease of fetal testosterone during the first half of gestation. In conclusion, the potential alteration of placental gonadotrophic stimulation caused by changes in GnRH release (produced by the effects of VA on GABA) cannot be ruled out at present, at least as a possible pathogenic mechanism in the association between maternal treatment with VA and hypospadias in the newborn.

Conclusion

The magnitude of the risk of developing hypospadias after prenatal exposure to VA is 5.71 times higher than in non-exposed infants. If we assume that the frequency of hypospadias in the population is as high as 1.8/1000 births,[18] then the risk of having an infant with hypospadias is 1 case in every 97 women treated with VA. This information is of great importance for risk assessment, not only because it is more accurate but also because unlike other major congenital malformations associated with VA (i.e. spina bifida, congenital heart defects, limb anomalies and others), hypospadias cannot be easily detected by current prenatal diagnostic techniques.[14]

Acknowledgements

This work was supported by a grant from the Fundación Raul González-Salas (a Non Governmental Organization to promote Science and Culture), Spain. The authors have no conflicts of interest that are directly relevant to the content of this study.

The ECEMC Working Group that participated in this project in 2005 by Spanish region are as follows. Andalucía: Barcia JM (Cabra), Cózar JA (Huércal Overa), Esteban MV (Jaén), Fernández E (Antequera), Gallardo FL (Montilla), Gomar JL (La Línea), Lara A (Ubeda), Rodríguez A (Motril), Tapia JM (Puerto Real). Aragón: Muñoz MS (Teruel), Calvo MJ (Barbastro), Oliván del Cacho MJ (Huesca). I. Baleares: Gómez F (Mahón), Jiménez F (Manacor). Canarias: López Mendoza S (Tenerife). Cantabria: Canduela V (Laredo), Gómez-Ullate J (Santander). Castilla-La Mancha: Félix V (Toledo), García A (Guadalajara), García MJ (Cuenca), Huertas H (Valdepeñas), Salvat F (Hellín), Sánchez C (Puertollano), Suay M (Cuenca), Vázquez MS (Albacete). Castilla y León: Aparicio P (Burgos), Burón E (Valladolid), Centeno F (Valladolid), Marugán VM (Zamora) Mousallem AG (Medina del Campo), Nieto C (Segovia). Cataluña: Alsina MN (Calella), Martínez S (Girona), Marco JJ (Lleida), Moral A (Terrassa), Rosal J (Tortosa), García MM (Figueres), Foguet A (Olot), Caubet I (Vielha), Puig I (Girona), Martí JJ (Santa Coloma de Gramanet), Serrano A (Mora d´Ebre), Torres JM (Palamós). Comunidad de Madrid: Conde JF (Madrid), Martín F (Madrid), Martínez MN (Leganés), Gallegos S (El Escorial). Comunidad Foral de Navarra: Egüés J (Pamplona). Comunidad Valenciana: Andrés M (Valencia), Beseler B (Denia), Climent S (Xátiva y Ontinyent), García C (Valencia), González de Dios J (San Juan de Alicante), Jovani C (Vila-Real), Martínez A (Requena), Pi G (Alzira), Sanchis A (Valencia), Vizcaíno C (Elche). Extremadura: Arroyo I (Cáceres), Galán E (Badajoz), Contreras J (Coria), Gómez H (Llerena), RotaL (Navalmoral de la Mata). Galicia: Blanco M (Vigo), Martínez MA (Vilagarcía de Arousa), Silveira M

(Burela). Principado de Asturias: Rodríguez MC (Riaño), Mayoral B (Cangas del Narcea), Suarez ME (Avilés). País Vasco: PaisánL (San Sebastián), Pérez JL (Basurto), Zuazo E (Zumárraga). Región de Murcia: Contessotto C (Santiago de la Ribera), Hernández F (Murcia), López JA (Lorca), Martín JM (Murcia), Peñas A (Yecla), Rubio MJ (Murcia). La Rioja: Garijo C (Calahorra).

References

1. Robert E, Guibaud P. Maternal valproic acid and congenital neural tube defects [letter]. Lancet 1982; 2: 937
2. Bjerkedal TA, Czeizel A, Goujard J, et al. Valproic acid and spina bifida [letter]. Lancet 1982; 2: 1096
3. Martínez-Frías ML, Rodríguez-Pinilla E, Salvador J. Valproate and spina bifida [letter]. Lancet 1989; 1: 611
4. Prieto L, Martínez-Frías ML. Case-control studies using only malformed infants: are we interpreting the results correctly? Teratology 1999; 60: 1-2
5. DiLiberti JH, Farndon PA, Dennis NR, et al. The fetal valproate syndrome. Am J Med Genet 1984; 19: 473-81
6. Jager-Roman E, Deichi A, Jacob S, et al. Fetal growth, major malformations, and minor anomalies in infants born to women receiving valproic acid. J Pediatr 1986; 108: 997-1004
7. Lammer EJ, Sever LE, Oakley Jr GP. Teratogen update: valproic acid. Teratology 1987; 35: 465-73
8. Ardinger HH, Atkin JF, Blackston RD, et al. Verification of the fetal valproate syndrome phenotype. Am J Med Genet 1988; 29: 171-85
9. Martínez-Frías ML. Clinical manifestation of prenatal exposure to valproic acid using case reports and epidemiologic information. Am J Med Genet 1990; 37: 277-82
10. Rodríguez-Pinilla E, Arroyo I, Fondevilla J, et al. Prenatal exposure to valproic acid during pregnancy and limb deficiencies: a case-control study. Am J Med Genet 2000; 90: 376-81
11. Bertollini R, Kallen B, Mastroiacovo P, et al. Anticonvulsant drugs in monotherapy: effect on the fetus. Eur J Epidemiol 1987; 3: 164-71
12. Winter RM, Donnai D, Burn J, et al. Fetal valproate syndrome: is there a recognisable phenotype? J Med Genet 1987; 24: 692-5
13. Lindhout D, Omtzigt JG, Cornel MC. Spectrum of neural-tube defects in 34 infants prenatally exposed to antiepileptic drugs. Neurology 1992; 42: 111-8
14. Bradaî R, Robert E. Diagnostic anténatal échographique chez la mère épileptique sous acide valproice. Etude rétrospective ou 161 cas de Registre France/Centre-Est de malformations congénitales. J Gynecol Obstet Biol Reprod 1998; 27: 413-9
15. Wyszynski DF, Nambisan M, Surve T, et al. Increased rate of major malformations in offspring exposed to valpoate during pregnancy. Neurology 2005; 64: 961-965
16. Arpino C, Brescianini S, Robert E, et al. Teratogenic effects of antiepileptic drugs: use of an international database on malformations and drug exposure [MADRE]. Epilepsia 2000; 41: 1436-43
17. The Centre of the ICBDSR. International Clearinghouse for Birth Defects Surveillance and Research (ICBDRS): annual report [online]. 2005; Rome. ISSN 0743-5703 Available from URL: http://www.icbdsr.org [2008 May 5]
18. Bermejo E, Cuevas L, Mendioroz J, et al. Anomalías congénitas en España: vigilancia epidemiológica en el último cuarto de siglo [1980-2004]. Boletín del ECEMC: Revista de Dismorfología y Epidemiología 2005; V (4): 62-85 [online]. Available from URL: http://bvs.isciii.es/mono/pdf/CIAC_04.pdf [Accessed 2008 May 5]
19. Prieto L, Martinez-Frias ML. Case-control studies using only malformed infants who were prenatally exposed to drugs: what do the results mean? Teratology 2000; 62 (1): 5-9
20. Martínez-Frías ML. Postmarketing analysis of medicines: methodology and value of the Spanish case-control study and surveillance system in preventing birth defects. Drug Safety 2007; 30 (4): 307-16
21. Martínez-Frías ML. Interviewer bias and maternal bias. Teratology 1993; 47: 531-2
22. Källén B. Valproic acid is known to cause hypospadias in man but not reduce anogenital distance or causes hypospadias in rats. Basic Clin Pharmacol Toxicol 2004; 94 (1): 51-4
23. Licht P, Harbarth P, Merz WE. Evidence for modulation of human chorionic gonadotropin [hCG] subunit messenger ribonucleic acid levels and hCG secretion by γ-aminobutyric acid in human first trimester placenta in vitro. Endocrinology 1992; 130: 490-6
24. Bhasin S, Heber D, Steiner BS, et al. Hormonal effects of gonadotropin-releasing hormone (GnRH) agonist in the human male. III: effects of long term combined treatment with GnRH agonist and androgen. J Clin Endocrinol Metab 1985; 60 (5): 998-1003

Correspondence: Dr *Elvira Rodríguez-Pinilla*, Instituto de Salud Carlos III, Sinesio Delgado 4-6, Pabellón 6, Madrid, CIAC, 28029, Spain.
E-mail: e.rodriguez-pinilla@isciii.es