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Compensatory Function of the Remaining Testis is Dissociated in Boys and Adolescents with Monorchidism

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Abstract

Objective. Compensatory hypertrophy has been classically described in patients with monorchidism. However, it remains unclear whether there is a functional compensatory activity of the different cell populations. Our aim was to assess the functional capacity of the solitary testis in monorchid males from infancy through puberty in order to determine whether the remaining gonad is capable of compensating the functional activity of Sertoli and Leydig cells of the absent gonad.

Design. In a retrospective, cross-sectional, analytical study performed at a tertiary paediatric public hospital, we included 89 boys with monorchidism and 358 healthy controls, aged 6 moths to 18 years. Testicular volume and circulating levels of reproductive hormones were compared between patients with monorchidism and normal boys. Serum AMH and FSH were used as biomarkers of the functional mass of prepubertal Sertoli cells, whereas serum testosterone and LH were used as biomarkers of Leydig cells.

Results. In the vast majority of the cases, the volume of the testis of monorchid boys was smaller than the sum of the volume of both testes of healthy controls. Serum AMH was lower and FSH was higher in patients with monorchidism than in controls aged <3 years and >13 years. Serum testosterone and LH did not differ significantly between patients and controls.

Conclusions. In boys and adolescents with monorchidism, there is a dissociated capacity of the remaining testis to compensate for the absence of the other gonad: while Leydig cell function is largely compensated, Sertoli cell proliferation and function is lower than in controls.

Introduction

Compensatory hypertrophy of a paired organ is a frequent observation when one of the organs is hypotrophic or absent. Compensatory testicular hypertrophy was first described by Laron and Zilka in patients with unilateral cryptorchidism [1], and is typically described in males with monorchidism. Size enlargement of the scrotal gonad has even been proposed to be predictive of the absence of the contralateral testis [2-4]. Yet, hypertrophy of the persistent organ does not guarantee full compensation of paired organ function. Monorchidism can be congenital [5], or acquired as a consequence of different insults such as infection, testicular torsion and orchiectomy due to testicular tumours or testicular atrophy after orchiopexy. Its prevalence rate is 0.02% in newborn boys [6] and 1.7% to 4% in cryptorchid boys [6, 7]. The mechanism by which the compensatory hypertrophy occurs is not known. Some studies suggest that compensatory hypertrophy depends on factors such as age of onset of anorchidism and functional state of the present testes [2].

The male gonad has two distinctive functional compartments, which evolve differently through postnatal development: the seminiferous tubules, containing Sertoli and germ cells, and the interstitial tissue, where lie the Leydig cells [8]. Sertoli cells represent the major proportion of testicular volume before puberty [9]. During the active period of the pituitary-gonadal axis taking place in the first 3-6 months of postnatal life [10-12], FSH provokes Sertoli cell proliferation and boosts the secretion of anti-Müllerian hormone (AMH) [13] and of inhibin B [14], whereas LH induces Leydig cell androgen production. Afterwards, pituitary gonadotropin levels decline and persist low during childhood. Leydig cells dedifferentiate and androgen production drops to undetectable amounts, and germ cell activity is arrested at the pre-meiotic stage. Although there is a waning in Sertoli cell proliferation, they remain functionally active, as reflected by their production of AMH [9] and inhibin B [15]. At the age of pubertal onset, the increase in gonadotropins induce testosterone production, which results in the maturation of the seminiferous tubule populations: Sertoli cell AMH secretion declines at the time germ cells undergo the full spermatogenic process leading to the overt increase in testicular volume and to sperm production.

The reproductive aptitude of monorchid males has been reported in adults [16, 17], but the functional capacity of the solitary testis has received little attention in the paediatric population, with its assessment having relied mainly on the measurement of indirect markers of testicular function, like serum gonadotropins [7, 18], which lacks adequate sensitivity [19]. Therefore, it remains unclear whether scrotal testis hypertrophy can functionally compensate the activity of the different cell populations of the missing gonad [6;7]. The aim of this study was to assess the functional capacity of the solitary testis in monorchid males from infancy through puberty in order to determine whether the

remaining gonad is capable of compensating the functional activity of Sertoli and Leydig cells of the absent gonad. Secondarily, we analysed whether AMH levels are associated with the degree of compensatory hypertrophy in prepubertal boys with monorchidism. We studied a large cohort of 89 patients with monorchidism and compared them with 358 healthy controls in terms of circulating levels of reproductive hormones. Serum AMH and testosterone were used as direct biomarkers of Sertoli [15] and Leydig cell function, respectively, whereas FSH and LH were used as indirect biomarkers. Hitherto, there is no effective method to certify the existence or the absence of a non-palpable gonads. Both ultrasound and MRI have low sensitivity in the identification of abdominal testes [20-22]. Laparoscopy, the most commonly used procedure, may also have false negative results [23]. In the quest for a non-invasive test that could circumvent surgery, we also analysed whether AMH levels could be useful to certify the absence of the non-palpable testis.

Subjects and methods

Study design

This study followed a retrospective, cross-sectional, analytical design, and was performed at the Division of Endocrinology of the Ricardo Gutiérrez Children's Hospital, a tertiary paediatric public hospital in Buenos Aires, Argentina.

A careful review of history charts was performed by the same paediatric endocrinologist. Surgical and clinical characteristics, including testicular volume measured by comparison with Prader's orchidometer [24], pubic hair and genital development according to Marshall and Tanner [25], and hormonal values were extracted from the history chart.

Patients

Patients with monorchidism. All clinical charts of subjects evaluated at the Division of Endocrinology of the Ricardo Gutiérrez Children's Hospital between 1997 and 2012, and encoded in our database with the diagnosis of monorchidism, were reviewed. Monorchidism was defined by the absence of one testis, as verified by surgical exploration. Patients whose history chart was incomplete, and those with disorders of sex development, hypogonadotropic hypogonadism or genetic syndromes that can affect testicular function were excluded.

Healthy controls. Between January 2007 and December 2009, 358 apparently normal males with no history of endocrine or urologic disorders, aged 2 days to 18 years, attending the Central Laboratory of the Ricardo Gutiérrez Children's Hospital were recruited to establish reference values for serum LH, FSH, testosterone and AMH, as previously described [26].

Outcome measures and definitions

Circulating levels of reproductive hormones were compared between patients with monorchidism and normal boys. Serum AMH and FSH were respectively used as direct and indirect biomarkers of the functional mass of prepubertal Sertoli cells [15], whereas serum testosterone and LH were respectively used as direct and indirect biomarkers of Leydig cells.

In prepubertal patients, to determine whether AMH levels reflect the degree of compensatory hypertrophy in boys with monorchidism, we evaluated the correlation between serum AMH and testicular volume. This analysis was limited to boys <9 years-old, because after the onset of puberty testicular volume is inversely correlated with the levels of AMH due to the inhibitory effect of

androgens on Sertoli cell [13, 27]. Only the first AMH measurement available for each patient was included in the cross-sectional analysis. To evaluate if AMH levels are useful to certify the absence of the non-palpable testis in prepubertal boys, we performed a ROC curve analysis considering boys with monorchidism as cases and healthy boys as controls.

Pubertal onset was assumed only when testicular volume increase was accompanied by secondary sexual characteristics, rather than by the sole occurrence of testicular volume ≥4 ml, since compensatory hypertrophy in prepubertal children may result in testicular volume beyond 4 ml. For the primary analysis, patients with monorchidism and controls were grouped by age intervals. A secondary analysis was performed according to Tanner stage.

To determine if the existence of compensatory hypertrophy depend on the age at which the monorchidism was established, we compared the existence and degree of compensatory hypertrophy between children with congenital and those with acquired monorchidism. Compensatory hypertrophy was defined by the existence of testicular volume >2 ml in prepubertal boys or >25 ml in pubertal boys [4].

The sample size was calculated for the main outcome measure, i.e. the comparison between AMH levels in patients with monorchidism and healthy control boys <9 years-old. The estimated study size required to incorporate 55 boys in each group in order to detect a difference of at least 30% in serum AMH levels between monorchid and control boys, with a power of 80% and an α error of 5%.

Hormone assay methods

AMH: AMH was determined using an enzyme-linked immunoassay specific for human AMH (EIA AMH/MIS®, Beckman-Coulter Co., Marseilles, France), as previously validated by our group [19, 26]. Intra- and interassay coefficients of variation were, respectively, 10.5% and 9.4% for a serum AMH concentration of 700 pmol/L, and 11.1% and 12.8% for a serum AMH concentration of 7 pmol/L.

Gonadotropins: LH and FSH were determined using electrochemiluminescent immunoassays (ECLIA, Roche Diagnostics GmbH, Mannheim, Germany) as described [19]. Intra- and inter-assay coefficients of variation were 1.1% and 1.8% for LH, respectively, for a mean LH concentration of 2.8 IU/L and 1.4% and 1.5% for a mean LH concentration of 16.9 IU/L. Intra- and inter-assay coefficients of variation were 1.0% and 4.2% for FSH, respectively, for a mean FSH concentration of 14.8 IU/L and 1.1% and 4.1% for a mean FSH concentration of 23.4 IU/L. When serum LH or

FSH levels were undetectable, the value of the limit of quantification (functional sensitivity) was attributed.

Testosterone: Testosterone was determined in serum using an electrochemiluminiscent immunoassay (ECLIA, Roche Diagnostics GmbH, Mannheim, Germany) as described [19]. Intra- and inter-assay coefficients of variation were 2.4% and 2.6%, respectively, for a mean testosterone concentration of 176 ng/dl (6.10 nmol/L) and 1.2% and 2.3% for a mean testosterone concentration of 455 ng/dl (15.78 nmol/L).

Statistical analyses

Data distribution was assessed for normality using the Shapiro–Wilk test. Results are expressed as median and interquartile range. Because non-Gaussian distribution was found in most cases, nonparametric tests were used for comparisons. Mann–Whitney test was used to compare serum hormone levels between two independent groups. Fisher's exact test was used to compare categorical variables. The correlation coefficient between testicular volume and serum AMH in patients with monorchidism was calculated using the nonparametric Spearman's test. The level of significance was set at $P < 0.05$. All statistical analyses were performed using Graphpad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

Ethical issues

The study protocol was approved by the Institutional Review Board and Ethics Committee of the Ricardo Gutiérrez Children's Hospital. Because the study of patients with monorchidism was based on a retrospective clinical chart review with descriptive purposes and no anticipated effect on prognosis or therapeutic management of the patients whose charts were included, the need for a written informed consent was waived. For the control group, written informed consent was given by the participant's parents, and assent was given by the participants over 7 years of age.

Results

Characteristics of the study population

Out of 119 eligible cases (Figure 1), 89 patients with monorchidism aged 1.1 to 18.7 years were included in the analysis. Median age at first visit was 5.1 years (range 0.3-14.5 years). A total of 168 serum samples were assessed, since follow-up was available in 83 of the 89 patients, with a median follow-up of 7.3 years (range 0.4 to 17.3 years). The prevalence of left monorchidism, i.e. absence of the right testis, was 48 cases (54%) in our series. The occurrence of acquired monorchidism was ascertained in 44 patients (49%): orchiectomy due to testicular tumour in 7 cases and to atrophic testis with testicular-epididymal dissociation in 4, atrophy following orchiopexy in 20, testicular torsion in 11, mumps orchitis in 1 and trauma in 1. In the remaining 45 patients, only one testis was palpable at birth, and an acquired aetiology for monorchidism could not be evidenced, hence congenital monorchidism was suspected.

Monorchidism in boys <9 years-old

In prepubertal age (<9 years-old), 59.5% of boys with monorchidism presented moderate hypertrophy of the remaining gonad. The volume of the solitary testis of monorchid children was not significantly different from that of the largest testis of healthy controls aged 6 months to 2.9 years, but it was bigger in monorchid boys than in controls aged 3 to 8.9 years. However, the volume of the testis of monorchid boys was smaller than the bi-testicular volume of controls in both subgroups (Table 1), indicating that testicular hypertrophy did not fully compensate the tissue mass of two gonads.

In concordance, median serum AMH was significantly lower in patients with monorchidism than in age-matched controls (Table 1). Testicular volume correlated significantly with AMH in boys <9 years (Figure 2), showing that AMH levels reflect the degree of hypertrophy in prepubertal patients with monorchidism. AMH levels were below the normal range in 4 out of 6 cases in the monorchid boys aged <3 years and in 13 out of 39 cases in the 3-8.9 year-old subgroup (Figure 3). To test whether the absence of one testis during the postnatal activation period of the hypothalamic-pituitary-gonadal axis could elicit a greater compensatory response, we analysed separately patients with congenital monorchidism. In the 6 months-2.9 years group, boys with congenital monorchidism (median age: 1.5 yr, IQR: 1.2-2.0 yr) had a lower serum AMH (median 324 pmol/L, IQR: 204-939 pmol/L) than healthy controls (median age: 1.9 yr, IQR: 1.0-2.4) in whom AMH was 1067 (IQR: 807-1460) pmol/L (two-tailed Mann-Whitney U 17.0, P=0.008). Similarly, in the 3.0-

8.9 years group, boys with congenital monorchidism (median age: 5.9 yr, IQR: 4.2-7.4 yr) had a lower AMH: 465 (IQR: 180-641) pmol/L than controls (median age: 5.4 yr, IQR: 4.3-7.0 yr) 596 (IQR: 420-873) pmol/L (two-tailed Mann-Whitney U 693.5, *P*=0.024).

To rule out the possibility that AMH was lower in patients with monorchidism because the remaining testis was not normal, we analysed a subset of 13 monorchid patients with no history compatible with damage of the remaining testis, i.e. monorchidism due to surgical removal of one testis following testicular torsion, traumatism or tumour (Figure 3). Although the sample size was limited, serum AMH was low in 9 of them (69.2%), suggesting that one testis with no overt history of defect is unable to fully compensate Sertoli cell function.

In order to evaluate if AMH level was useful in prepubertal boys to certify the existence of only one gonad, we performed a ROC curve analysis, comparing monorchid boys as cases and healthy boys as controls. Area under the ROC curve was 0.772 (95% confidence interval 0.687 to 0.856), and the best cut-off value (AMH 400 pmol/L) had very low sensitivity (52.4%; 95% CI 36.4 to 68.0%) and insufficient specificity (89.8%; 95% CI: 83.7 to 94.2%) to diagnose monorchidism.

Median serum FSH was moderately increased in boys with monorchidism below the age of 3 years, i.e. just after the postnatal activation of the pituitary-gonadal axis, but not during the rest of childhood. Testosterone and LH were within the normal range in patients with monorchidism <9 years-old (Table 1 and Figure 3).

Monorchidism in boys older than 9 years-old

In patients with monorchidism aged 9 years or older, the volume of the present testis was >25ml in 20% of the cases. From the age of 13 years onwards, i.e. when patients were in the most advanced stages of pubertal development, the size of the solitary testis of monorchid boys was significantly bigger than the largest testis of healthy controls, but it did not reach the normal bi-testicular volume (Table 2), indicating that testicular hypertrophy did not fully compensate the tissue mass of two gonads.

AMH levels were significantly lower, and FSH were significantly higher in patients with monorchidism (Table 2 and Figure 3), indicating that the seminiferous tubule compartment of the solitary testis was unable to fully compensate the function of the absent gonad. Conversely, LH and testosterone showed no significant differences between patients with monorchidism and healthy controls, showing that the interstitial tissue of the solitary testis was capable of compensating the androgenic function.

Discussion

The results of this survey, which included 89 patients with monorchidism spanning infancy, childhood and puberty, indicate that there is a dissociated capacity of the remaining testis to fully compensate for the absence of the other gonad: while Leydig cell function is largely compensated, Sertoli cell proliferation and function is insufficient. Indeed, testosterone and LH levels were normal during pubertal development in the vast majority of patients with monorchidism, in line with previous results in a small series of 11 patients [28]. Conversely, lower AMH and higher FSH in monorchid boys indicate that the remaining testis does not fully compensate the function of the absent one. Furthermore, the volume of the testis is primarily dependent on the mass of Sertoli cells in prepuberty; after puberty, testicular volume is determined by germ cell numbers, which is limited by the peak number of Sertoli cells reached at during infancy and childhood [29]. In both prepubertal and pubertal patients with monorchidism of this study, although the volume of the testis was larger than the mean volume of the two gonads of healthy controls, it did not reach the double of a normal testis, thus indicating that the number of Sertoli cells of the remaining gonad was insufficient to fully compensate the absence of the second testis. This is in concordance with low inhibin B levels observed in a small series of boys [28] and with oligospermia reported in adult males [16] with monorchidism. For ethical reasons, semen analysis was not performed in our patients; therefore, we cannot guarantee that insufficient testicular volume compensation resulted in oligospermia or impaired fertility outcome.

In patients with unilateral cryptorchidism or monorchidism, testis hypertrophy and functional compensation by the scrotal gonad is believed to be dependent on three factors: the magnitude of functional reduction of the testicular parenchyma of the affected gonad, the age at injury and the status of the descended testis. Congenital monorchidism and acquired monorchidism may occur in patients in whom a primary testicular disorder affecting both testes could be suspected, for instance in the testicular regression syndrome [6, 7] and in patients with a history of unilateral or bilateral cryptorchidism [1, 17]. In these cases, the remaining testis may be dysfunctional and, thus, inept for functional compensatory hypertrophy. The lower AMH levels observed in our patients with monorchidism are most probably not due to a primary defect of the present testis, at least in the subset of cases in whom a history compatible with primary gonadal dysfunction could be ruled out.

The precise mechanism underlying the enlargement of the remaining testis in monorchid patients is not fully understood. The effect of increased FSH levels is suspected to be at least partially responsible for Sertoli cell hyperplasia. Compensatory hypertrophy would then be more likely in

patients with congenital monorchidism, in whom the early postnatal activation of the hypothalamic-gonadotrope axis would be exaggerated [19]. In the present study, we could not demonstrate any compensatory function in patients with congenital monorchidism.

The major strength of this work is that we analysed a large series of patients with certified monorchidism during childhood by assessing serum AMH, a validated marker of testicular function with no need for stimulation tests. Indeed, while the gonadotrophs and Leydig cells are functionally quiescent in boys between infancy and puberty, Sertoli cells remain active and secrete huge amounts of AMH. Therefore, serum AMH is a widely accepted biomarker of testicular activity during childhood [30-36]. Furthermore, serum AMH has been postulated as a surrogate marker for the mass of functional Sertoli cells in patients with gonadal dysgenesis [37], hypogonadotrophic hypogonadism [14, 38] and Sertoli cell hyperplasia [39, 40]. In the present work, the significant correlation between testicular volume and AMH suggested that AMH levels reflect the degree of compensatory hypertrophy in prepubertal patients with monorchidism.

Due to its retrospective design, our study has some limitations: testis volume values obtained from the history charts had been measured by the patient's paediatric endocrinologist and not by only one observer, which may have resulted in less precise results owing to greater coefficients of variations than those obtained in a prospective study with only one or two observers. Also, testicular volume was obtained by comparison with Prader's orchidometer rather than by ultrasound measurements, which gives more accurate results. Yet, even if less accurate, measurements by the Prader's orchidometer show a strong correlation with ultrasound measurements [41]. Another limitation of this study is that comparisons of reproductive hormone levels between patients and controls >9 years-old were made according to age and not Tanner stage. The reason is that testicular volume is one of the most important features considered to distinguish between Tanner stages G2 and G3 [25], and testicular hypertrophy expected to occur in patients with monorchidism precluded the use of gonadal volume to assess pubertal maturation. Pubertal development stages are extremely variable between individuals, mainly in boys aged 11 to 13 years. The large interindividual variations could explain the lack of significant differences in serum AMH between monorchid patients and healthy controls only in this age group.

It should be noted that the differences observed in the median levels of the markers of Sertoli cell functional mass, i.e. AMH and FSH, could be explained by the existence of a subset of patients with clearly abnormal values, while the remaining cases have normal levels. This suggests that two populations of patients with monorchidism may exist, including one with normal hormone levels

who subsequently may prove to have normal fertility and the other with elevated FSH levels who may attain a subfertile state later in life.

Because the normal range of serum AMH is relatively large, it did not prove useful in our study to certify the lack of a second gonad, e.g. in abdominal position, in our study population. We are aware that a limitation of our study is that in the ROC curve analysis, we compared serum AMH of monorchid patients with that of healthy controls, rather than with unilateral cryptorchid patients. However, the fact that serum AMH was unable to distinguish patients with monorchidism from healthy boys indicates that it would be less efficient to distinguish between patients with monorchidism and those with unilateral cryptorchidism.

In summary, patients with monorchidism show a dissociated capacity of compensation of testicular function: the interstitial compartment is capable to respond to LH increase with adequate testosterone production, thus avoiding hypoandrogenism, whereas Sertoli cells seem unable to fully compensate for the absence of the other gonad, probably resulting in lower total cell numbers when compared to that of two testes, and leading to a lower total testicular mass, a decreased AMH production, and high circulating FSH. Whether this may predict infertility needs to be addressed by studying patients with a sufficiently long follow-up, until adulthood.

Declaration of interest

RPG, PB and RAR have received honoraria from CONICET (Argentina) for technology services using the AMH ELISA. CH and SG have nothing to disclose.

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Author contributions

RPG, CH and SG reviewed the history charts of patients with monorchidism. RPG, SG and RAR recruited and performed the clinical assessment of healthy controls. PB managed the database and

validated the AMH assay. RPG, CH and RAR performed the analyses of the results. RPG and RAR wrote the manuscript. All authors approved the final version.

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Figure legends

Figure 1. Flow diagram of enrolment of patients with monorchidism.

Figure 2. Correlation between serum antimüllerian hormone (AMH) levels and testicular volume in prepubertal boys with monorchidism.

Figure 3. Serum reproductive hormone levels in boys with monorchidism and in healthy controls.

Table 1. Testicular volume and reproductive axis hormone levels in 45 boys with monorchidism and in 147 healthy controls aged <9 years. Results are expressed as medians (interquartile range) and were compared using the Mann–Whitney test.

	6 mo-2.9 years-old			3-8.9 years-old		
	Monorchidism	**Control**	***P***	**Monorchidism**	**Control**	***P***
Age	1.3 (1.1-1.7)	1.9 (1.0-2.4)		6.6 (4.4-7.7)	5.4 (4.3-7.0)	
Testicular Volume (mL)*	2 (0.5-3)	L: 2 (1-2) B: 4 (2-4)	0.735 **0.004**	3 (0.5-5)	L: 2 (1-3) B: 4 (2-6)	**<0.001** **<0.001**
AMH (pmol/L)	324 (221-820)	1067 (807-1460)	**0.0009**	403 (203-637)	596 (420-873)	**0.001**
FSH (IU/L)	1.10 (0.72-6.72)	0.63 (0.26-1.37)	**0.017**	0.99 (0.27-3.30)	0.79 (0.20-3.21)	0.106
Testosterone (ng/dL)	10 (10-21)	10 (10-10)	NA	10 (10-66)	10 (10-10)	NA
LH (IU/L)	0.07 (0.10-0.63)	0.10 (0.10-0.43)	0.580	0.10 (0.05-0.28)	0.10 (0.10-0.19)	0.519

* Testicular volume reflects that of the only testis in boys with monorchidism, and in healthy controls that of the largest testis (L) or that of the sum of both testes (B).

NA: statistical analysis was not applicable since testosterone levels were below the lower limit of detection of the assay in the vast majority of boys with monorchidism and in healthy controls.

Table 2. Testicular volume and reproductive axis hormone levels in 73 boys with monorchidism and in 155 healthy controls aged >9yr. Results are expressed as medians (interquartile range) and were compared using the Mann–Whitney test.

	9-10.9 yr			11-12.9 yr			13-14.9 yr			≥15 yr		
	Monorchid-ism	**Control**	***P***	**Monorchid-ism**	**Control**	***P***	**Monorchid-ism**	**Control**	***P***	**Monorchid-ism**	**Control**	***P***
Age	9.9 (9.4-10.4)	10.0 (9.4-10.6)		11.5 (11.3-12.2)	12.3 (11.8-12.6)		13.7 (13.4-14.1)	13.8 (13.4-14.3)		16.2 (15.8-16.9)	16.0 (15.3-16.7)	
Tanner stage	G1: 14 G2: 4 G3: 2 G4: 0 G5: 0	G1: 29 G2: 4 G3: 2 G4: 0 G5: 0		G1: 10 G2: 13 G3: 7 G4: 6 G5: 4	G1: 5 G2: 21 G3: 15 G4: 3 G5: 3		G1: 2 G2: 5 G3: 10 G4: 10 G5: 10	G1: 0 G2: 8 G3: 24 G4: 16 G5: 23		G1: 0 G2: 0 G3: 1 G4: 2 G5: 20	G1: 0 G2: 1 G3: 1 G4: 22 G5: 34	
Testicular Volume (mL)*	4 (1-25)	L:2 (2-8) B: 4 (4-14)	**0.027** 0.068	6 (2-25)	L: 8 (2-20) B: 16 (4-40)	0.598 **<0.001**	20 (5->25)	L: 15 (4-25) B 30 (9-50)	**0.001** **<0.001**	25 (6->25)	L: 20 (10-25) B: 40 (20-50)	**0.004** **<0.001**
AMH (pmol/L)	330 (196-446)	685 (402-905)	**<0.001**	165 (62-281)	257 (71-536)	0.134	48 (30-67)	72 (51-120)	**<0.001**	37 (20-44)	73 (51-113)	**<0.001**
FSH (IU/L)	2.20 (0.68-21)	1.59 (0.41-2.92)	0.168	2.78 (0.34-32.20)	2.79 (1.08-8.08)	0.821	4.30 (1.99-45.90)	2.72 (0.85-7.27)	**<0.001**	5.81 (1.90-63.90	3.21 (1.23-9.27)	**<0.001**
Testosterone (ng/dL)	10 (10-110)	10 (10-146)	0.058	26 (10-622)	111 (10-550)	**0.028**	253 (20-667)	280 (10-661)	0.429	424 (223-902)	467 (17-814)	0.426
LH (IU/L)	0.30 (0.10-1.80)	0.10 (0.10-3.04)	0.473	0.72 (0.05-7.40)	1.73 (0.10-3.57)	**0.002**	2.69 (0.43-17.40)	2.26 (0.42-8.99)	0.213	5.60 (1.40-24.40)	3.17 (1.12-7.52)	**0.007**

* Testicular volume reflects that of the only testis in boys with monorchidism, and in healthy controls that of the largest testis (L) or that of the sum of both testes (B).

Figure 1

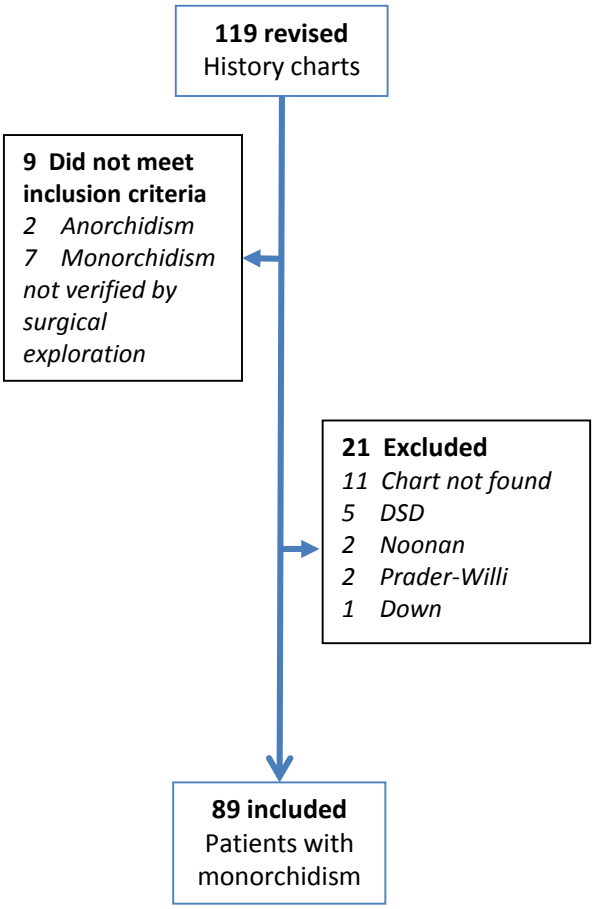

119 revised
History charts
9 Did not meet inclusion criteria
2 Anorchidism
7 Monorchidism not verified by surgical exploration
21 Excluded
11 Chart not found
5 DSD
2 Noonan
2 Prader-Willi
1 Down
89 included
Patients with monorchidism

Figure 2

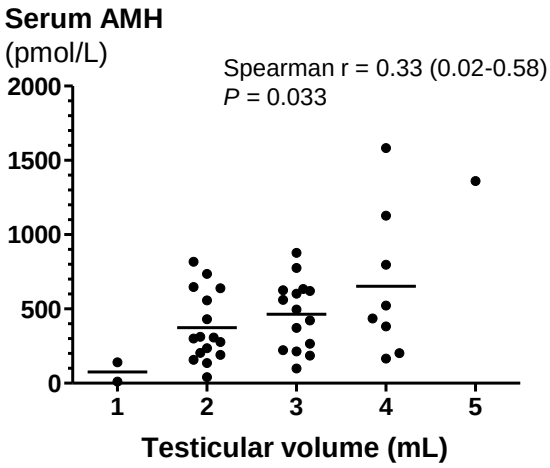
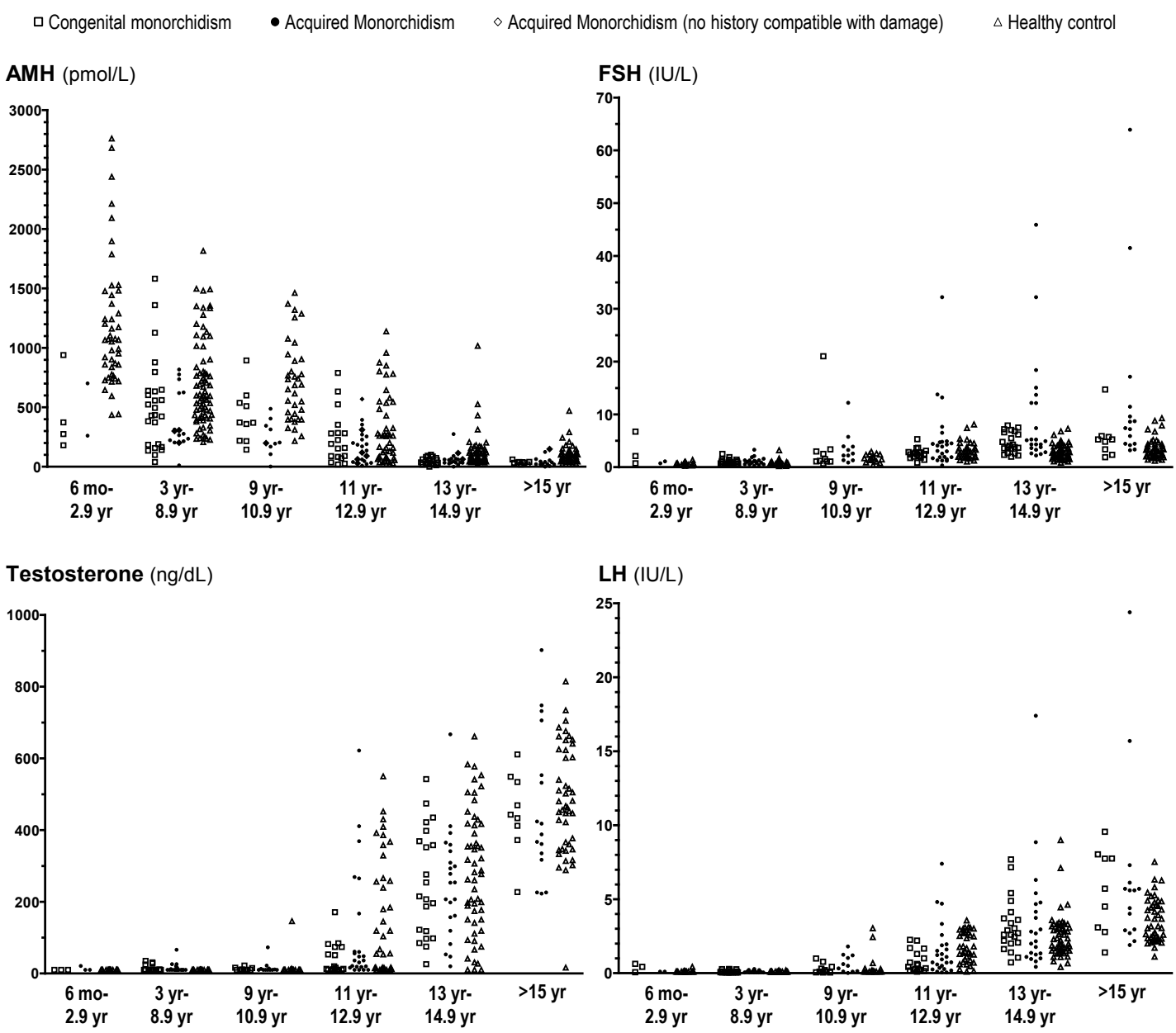

Serum AMH
(pmol/L)
Spearman r = 0.33 (0.02-0.58)
P = 0.033
2000
1500
1000
500
0
1
2
3
4
5
Testicular volume (mL)

Figure 3


□ Congenital monorchidism
● Acquired Monorchidism
◇ Acquired Monorchidism (no history compatible with damage)
△ Healthy control
AMH (pmol/L)
3000
2500
2000
1500
1000
500
0
FSH (IU/L)
70
60
50
40
30
20
10
0
Testosterone (ng/dL)
1000
800
600
400
200
0
LH (IU/L)
25
20
15
10
5
0
6 mo-2.9 yr
3 yr-8.9 yr
9 yr-10.9 yr
11 yr-12.9 yr
13 yr-14.9 yr
>15 yr