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ANTI-MULLERIAN HORMONE AND INHIBIN B AS A DIAGNOSTIC REPLACEMENT OF HUMAN CHORIONIC GONADOTROPIN STIMULATION TEST IN EVALUATION OF 46 XY DISORDERS OF SEX DEVELOPMENT

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Abstract

Background: As blood anti-Müllerian hormone (AMH) measurement may assess male gonad function without the need of invasive dynamic testing, it is becoming more and more common. A measurement of inhibin B may be thought of as a diagnosis tool that provides accurate data regarding the existence and functionality of the tests.

Objective: To assess the role of AMH and inhibin-B in diagnosis of 46, XY disorders of sex development (DSD) in comparison to HCG stimulation test.

Patients and methods: 42 individuals with 46 XY DSD were the subject of the research, which also included 42 healthy male participants from DEMPU, New Pediatric Hospital, and Cairo University. The analyzed patients had a 4 year average age.

Results: there was a statistically considerable variation between level of AMH in testicular agenesis/dysgenesis and 46 XY DSD group ($p<0.001$). Basal AMH and HCG-activated testosterone and DHT showed a substantial connection. Inhibin B, HCG-stimulated testosterone, and DHT were not shown to be substantially connected with one another. At a cut-off value of (43.2ng/ml), the sensitivity of AMH was (90.9%), specificity (96.8%), NPV (90.91%) PPV (96.77%), with overall accuracy (95.24%) to determine if functioning testicular tissue is present. While the inhibin B showed no discrimination between subjects who had functioning testicular tissue from those who had not.

Conclusion: AMH is valuable reliable non-invasive parameter for identifying functional testicular tissue in 46 XY DSD patients.

Key words: 46 XY DSD, inhibin B, anti-Mullerian hormone (AMH), testosterone, inhibin B,.

INTRODUCTION

Congenital abnormalities known as disorders of sex development (DSD) cause unusual gonadal, chromosomal, or anatomical sex development. **(Babbar and Shah, 2019).**

The following language was suggested by the 2005 Chicago Consensus, the Pediatric Endocrine Society (PES), and the European Society for Pediatric Endocrinology (ESPE): It has been suggested that DSD should take the place of words like intersex, pseudo-hermaphroditism, and sex reversal. **(Bennecke et al., 2020 and Majumdar et al., 2018).**

According to karyotyping, DSD includes a wide range of medical disorders that are categorized as 46, XX DSD, 46, XY DSD, and sex chromosome DSD. **(Hafez et al., 2014).**

Human chorionic gonadotropin (hCG) stimulated tests, which represents the active function of the testicular interstitial Leydig cells, has historically been used to evaluate the testicles in males. **(Rey et al., 2011)**. The effects of hCG have been shown to be more complex than first believed; in adding to abruptly increasing testicular T synthesis, hCG may also briefly create a condition of steroidogenic refractoriness to gonadotrophin stimulating that depends on both time and dosage. **(Lucas-Herald et al., 2020).**

Initial values of testosterone and gonadotropin in juvenile patients may not be helpful in determining testicular function. Serum anti-Müllerian hormone (AMH) quantification is becoming more common because it not only reveals the activity of FSH and androgens within the testis but also gives details about the behavior of the male gonad without the requiring for invasive dynamic tests, which are expensive and have negative side effects. **(Grinspon et al., 2012)**. During fetal life until the start of puberty, Sertoli cells release large quantities of AMH. Gonadotropins or sex steroids are not necessary for basal AMH expression, however FSH further boosts and testosterone decreases AMH synthesis. **(Lasala et al., 2004).**

Inducing Sertoli cell development during puberty, testosterone outperforms FSH in regulating AMH. As a result, AMH production declines. Patients with congenital or acquired anorchidism, as well as those with full gonadal dysgenesis, have undetectable serum AMH levels. **(Josso and Lee et al., 1997).**

When some individuals have cryptorchidism, partial gonadal dysgenesis, monorchidism, or central hypogonadism, for example, low serum concentrations of AMH may indicate intrinsic testicular dysfunction. **(Young et al., 2005)**.

The Sertoli cells release Inhibin B after being activated by gonadotropins. Male fetuses have detectable amounts of inhibin B in their blood, which slightly rise at term in neonates, stay high for 2-4 months, and then start to diminish. During puberty, serum inhibin B levels reach adult levels. **(Grinspon et al., 2012).** Basal inhibin B assessment may be thought of as a diagnosis tool that provides accurate data about both the existence and functioning of the testes since inhibin B was formerly thought to be the first relevant marker showing the existence of normal testicular tissue. **(Freire et al., 2018).**

SUBJECTS AND METHODS

The Diabetes Endocrine and Metabolism Pediatric Unit (DEMPU) outpatient's clinic at the New Children's Hospital, Faculty of Medicine, Cairo University, was the setting for this research, which included 42 individuals with a diagnosed 46 XY DSD.

The inclusion criteria: Patients presented with abnormal or ambiguous external genitalia, karyotyping: 46 XY (46 XY DSD), Patient age range: from the first day of birth to 15 years.

The exclusion criteria: DSD cases that are not 46 XY DSDs based on karyotyping. The current study also included 42 male age-matched controls who visited the hospital for minor diseases like the

conjunctivitis, common cold, and constipation. They were recruited through the pediatrics department's outpatient clinic. All subjects and controls signed consent forms. Approval was taken from the medical ethics committee of Cairo University.

All patients were subjected to: recording history, exams, both general and clinical, analysis of karyotypes, measures of hormones, Gonadal biopsies, laparoscopy, and abdominal-pelvic ultrasound were done for some patients at Abo El rish hospital and data were collected.

Statistical analysis:
The sample size is calculated by using software (the survey system) through entering the confidence interval CI 95% and margin error.

The collected data were tabulated and statistically analyzed. The variables were analyzed utilizing SPSS Statistics version 17 (SPSS Inc., Chicago, IL). The D'Agostino-Pearson test was used to determine if the dispersion of numerical data was normal. One-way analysis of variance was used to examine intergroup variations (ANOVA) for numerical parameters that were provided as mean ±SD (ANOVA). The median and interquartile range were utilized to depict non-parametric numeric values. The fisher's exact test was utilized to assess intergroup variations for categorical data that were reported as numbers and percentages.

The odds ratio (OR) with a 95% confidence interval (95% CI) for trend was utilized to compare categorical data, with a P-value of 0.05 being deemed statistically relevant. To evaluate the value of employing the hormone concentrations (AMH and inhibin B) as indicators for the existence of testicular tissues, receiver operating characteristic (ROC) curves were created. The trade-off between sensitivity and specificity when the cut-off changes for a particular indicator is shown by the ROC curves. An indicator for the total prediction ability of the marker is provided by the area under the curve (AUC)**.**

RESULTS

Table (1): Age-based sex rearing of 42 individuals with 46 XY DSD

Age group	**No of subjects (%)**	**Sex of rearing at birth**		**Reassigned sex**	
		No of subjects		**No of subjects**	
		Male	**Female**	**Male**	**Female**
Infant (0-2 years)	15 (35.7%)	11	4	12	3
Childhood (2-12 years)	24 (57.1%)	21	3	24	0
Adolescence (12-15 years)	3 (7.1%)	3	0	3	0
Total	42 (100%)	35	7	39	3

Out of 42 patients with 46 XY DSD, thirty-five cases (83.3%) were assigned at birth and raised as males, and seven cases (16.6%) were assigned at birth as females, four of them were re-assigned as males (9.5%), these four cases were as follows: one case diagnosed by biopsy as 46XY ovo-testicular disorder, two cases provisionally diagnosed as PAIS and one case as 5-α reductase deficiency. Three cases will continue as females (7.1%) provisionally diagnosed as CAIS. A gender evaluation committee made comprised of pediatric endocrinologists, urologists, pediatric surgeons, psychiatrists, and medical geneticists assigned female or male sex to each child in the series. This information is condensed in.

Table (2): The levels of testosterone, ▲4 androstenedione and dihydrotestosterone in the 46 XY DSD patients before and after Human Chorionic Gonadotropin (HCG) test:

Hormone	Before HCG test Cases (n=39)	After HCG test Cases (n=39)	p-value
Testosterone (ng/ml) • Median • Min-Max	 0.10 0.03– 11.20	 1.1 0.03–7.9	 < 0.001
▲4 androstenedione (ng/ml) • Median • Min-Max	 0.10 0.10 – 2.10	 0.30 0.10 – 1.70	 0.067
Dihydrotestosterone (DHT) (ng/dl) • Median • Min-Max	 0.95 0.1 – 38.0	 7.0 0.3 – 50.0	 < 0.001

The Table shows average and range of basal and post HCG testosterone, ▲4 androstenedione and dihydrotestosterone (DHT). The average basal value of testosterone was 0.1ng/ml, of ▲4 androstenedione was 0.1 ng/ml and of DHT before HCG test was 0.95ng/dl. Following HCG stimulation test a statistically substantial enhance was observed in testosterone and DHT as median testosterone level rose to 1.1 ng/ml and DHT was 7.0ng/dl ($p < 0.001$ in the two hormones).

Table (3): Assessment of AMH and inhibin B among the testicular dysgenesis/agenesis subgroup and the control group

Hormone	Testicular dysgenesis/ agenesis(n=13)	Control (n=42)	p-value
***AMH (ng/ml)** • Median • Min-Max	 26.7 0.05 – 69.7	 89.0 4.50-490	 <0.001
Inhibin B (pg/ml) • Median • Min-Max	 101 61.00 – 398	 148.5 61.00 –1011	 0.241

The Table shows the average measurement of AMH in the testicular dysgenesis/agenesis subgroup which was 26.7ng/ml while it was 89ng/ml in the control group with substantial difference between the two groups ($p < 0.001$). In contrast, the median measurement of inhibin B in the testicular dysgenesis/agenesis subgroup was 101pg/ml and in the control group was 148.5pg/ml with no statistical difference ($p= 0.241$).

Table (4): Assessment of Anti-Mullerian hormone (AMH) and inhibin B in the subgroup testicular dysgenesis compared with other subgroups of the 46 XY DSD patients (AIS, 5α-reductase deficiency, HH and ovotesticular DSD)

	Testicular dysgenesis/Agenesis (n=13)	46 XY DSD cases (AIS, 5α-reductase insufficiency, HH and Ovotesticular) (n=29)	p-value
***AMH (ng/ml)** • Median • Min-Max	 26.70 0.05 – 69.7	 84.00 1.40-490	 <0.001
Inhibin B (pg/ml) • Median • Min-Max	 101.00 61.00 – 398.00	 127.00 61.00 –464.00	 0.822
Basal testosterone (ng/ml)			

• Median • Min-Max	0.1 0.03-0.1	0.1 0.03-11.2	0.48
Stimulated testosterone (ng/ml) • Median • Min-Max	 0.2 0.03-0.7	 1.6 0.1-7.9	 <0.001
Stimulated DHT (ng/dl) • Median • Min-Max	 2.5 0.3-13.1	 9.35 0.9-50	 <0.001

The Table shows the median measurements of AMH in testicular dysgenesis/agenesis subgroup was 26.7ng/ml which was statistically substantially reduced than the median AMH levels of the other subgroups of the 46 XY DSD patients (AIS, 5α-reductase insufficiency, HH and ovotesticular DSD) 84ng/ml (p = <0.001).However, the median level of inhibin B in testicular dysgenesis/agenesis subgroup was 101 pg/ml and in the other subgroups was 127 with no statistical variation between both groups (p = 0.822).The mean level of basal testosterone was 0.1ng/ml in testicular dysgenesis/agenesis and also 0.1 in the other subgroups with no statistical variation between both groups (p = 0.48),On the other hand stimulated testosterone and stimulated DHT show significant variation between both subgroups (p <0.001).

Table (5): Correlation between Anti-Mullerian hormone (AMH) and other hormones among the patients' group:

Correlation between AMH and other hormones	**r-value**	**p-value**
AMH – HCG stimulated testosterone	0.571	<0.001
AMH - HCG stimulated dihydrotestosterone (DHT)	0.452	0.004
AMH – HCG stimulated ▲4 androstenedione (▲4A)	0.317	0.053
AMH – inhibin B	0.15	0.342

The Table shows the connection between AMH and other hormones among the patients' group. very substantial connections were found between AMH and HCG stimulated testosterone (r= 0.571; p < 0.001) Figure (20), HCG stimulated DHT (r= 0.452; p =0.004) Figure (21).
As opposed to that, there were no substantial connections between AMH and inhibin B levels (r = 0.15; p =0.342) Figure (22) or HCG stimulated ▲4 androstenedione (r =0.317; p =0.053).

Table (6): evaluation of inhibin B (in pg/ml) in each age group of the control group

	Control (n=42)	
Age group	**N**	**(%)**
Infant • Median(pg/ml) • Min-Max	15 158.00 61.00 – 1011.00	35.7%
Childhood • Median(pg/ml) • Min-Max	20 152.00 65.50 – 539.00	47.60%
Adolescence • Median(pg/ml) • Min-Max	7 84.00 68.00 – 279.00	16.70%

The Table shows the median and the range of inhibin B in each age group of the normal control, in the infancy the median was 158pg/ml and the range was (61-1011). In childhood control group, the median inhibin B value was 152 pg/ml and the range was (65.5-539). Also the mean of inhibin B in normal control adolescence group was 84 pg/ml and the range was (68-279) showing overlap in the

normal range of inhibin B between the three age group, Figure (16) shows values of inhibin B in different age groups of the controls.

Table (7): connections between inhibin B and other hormones among the patients' group

connections between inhibin B and other hormones	r-value	p-value
Inhibin B – HCG stimulated testosterone	0.075	0.649
Inhibin B-HCG stimulated dihydrote-stosterone (DHT)	0.198	0.226
Inhibin B–HCG stimulated ▲4 androste-nedione (▲4A)	0.074	0.660
Inhibin B- anti-Mullerian hormone (AMH)	0.150	0.342

The Table shows the connections between inhibin B and other hormones among the patients' group with no connections between them figure (23 and 24).

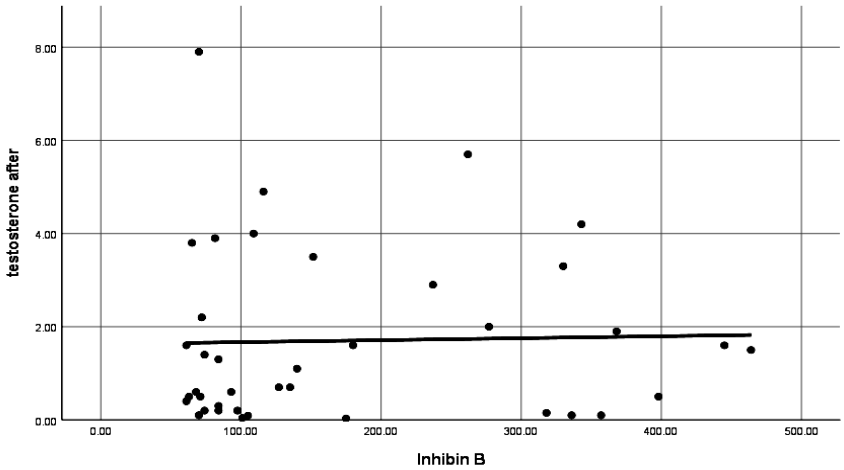

Figure (1): Connection between increased testosterone and inhibin B

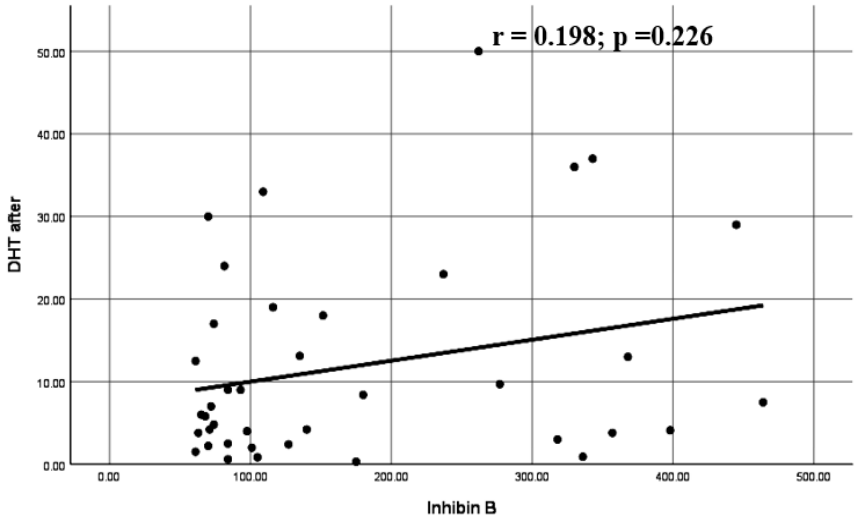

Figure (2): connections between inhibin B and stimulated DHT

DISCUSSION

Disorders of sexual development (DSD), are among the most fascinating conditions encountered by the clinician. Due to the complexity and heterogeneity of conditions under the 46, XY DSD umbrella, The ability to diagnose these conditions has been advanced rapidly through providing an interdisciplinary health care teams to provide care to patients and their families **(Buonocore et al., 2019).**

plasma inhibin B and AMH measures have served as helpful tools for clinical research in a variety of gonadal illnesses during the last ten years. **(Lahlou et al., 2009).**
In the present research, Age of presentation ranged widely, from 10 days to 13.5 years, with an mean age of 4 years. The percentage of the patients who were referred within the first two years of life were 35.7%, while 57.1% and 7.1% were referred in childhood period and adolescence period respectively. In Egypt, **Mekkawy et al., (2019)** diagnosed ten cases with DSD in the National Research Centre, their ages ranged between one month and 29 years. While in Nigeria **Ekenze et al., (2015)** performed an analysis of 39 children with DSD. average age was 3 years and the age range was from 2 months to 14 years.
Another study by **Hafez et al., (2014)** compared 43 healthy, males, age-matched controls to 43 patients of 46 with XY DSD. The age ranges were from 1 to 15 years and the percentage of the patients who were referred within the first two years of life were 37.2%, while 53.4% and 9.3% were referred in childhood period and adolescence period respectively.

The median level of stimulated testosterone in testicular dysgenesis/ agenesis cases was 0.2ng/ml, 1.9ng/ml in 5α-reductase cases and 1.8ng/ml in AIS group with substantial variation between them (p=<0.001). There was a substantial variation between testicular dysgenesis/agenesis group and 5α-reductase group (p=0.001) also between testicular dysgenesis/ agenesis group and AIS group (p< 0.001), while there was no substantial variation between 5α-reductase group and AIS group (p=1.0). The median level of stimulated DHT in testicular dysgenesis/agenesis cases was 2.5ng/dl, 7.5ng/dl in 5α-reductase cases and 18ng/dl in AIS with substantial variation between the three groups (p=<0.001), There was a substantial variation between testicular dysgenesis/agenesis group and AIS group only (p< 0.001).

In line with these results, **Ahmadifard et al., (2019)** in Iran concluded that the median stimulated testosterone in cases of AIS was 2.5ng/ml and the median stimulated DHT was 30.3ng/dl while the median testosterone level was 2.9ng/ml in 5α-reductase deficiency and stimulated DHT was 14.3ng/dl. Also **Ahmed et al., (1999)** found that in 23 of 30 infants with AIS testosterone was within age related reference ranges while seven cases were above this range.
In the present research the average measurement of AMH in testicular dysgenesis/agenesis group was 26.7ng/ml, in 5α-reductase group was 78.1 ng/ml and in AIS group was 90.75ng/ml, On comparing between testicular dysgenesis/agenesis group on one hand and 5α- reductase or AIS group on the other hand a substantial variation was found in both groups with p value 0.003 and < 0.001 respectively, while there was no substantial variation between 5α- reductase group and AIS (p =1).

In agreement with these findings a study by **Grinspon and Rey, (2010)** in Argentina discovered that anorchid patients' AMH levels were undetectable. Serum AMH was regular or elevated when only Leydig cells were impacted by hypogonadism. Patients with AIS also had normal or high values of AMH. Also, **Aksglaede et al., (2010)** in Denmark showed the same result.

This was also validated in a study by **Szarras-Czapnik et al., (2006)** 63 prepubertal patients from Poland with gonadal dysgenesis (n = 23), PAIS (n = 4), cryptorchidism (n = 26), and testicular agenesis (n = 10) had their blood AMH levels evaluated. AMH values were hardly detectable in males with testicular agenesis (average 0.14 ng/ml), but not affected in PAIS patients (median 49ng/ml).
In the present investigation the values of inhibin B were analysed in three age groups of controls. The value of the inhibin B in infant age group showed rise, the median measurement was 158pg/ml but its level slightly declined in childhood period where the median was 152pg/ml and in prepubertal age to become 84pg/ml with no significant difference between them.

Prior studies examined the amount of inhibin B in healthy males from infancy until adolescence, (**Lee et al., 2010; Ohnuma et al., 2007; Crofton et al., 2002; Raivio and Dunkel, 2007; Andersson and**

Skakkebaek; 2001 and Bergadá et al., 2001). They all came to the same conclusion: Inhibin B levels were high in young boys, dropped progressively between the ages of 6 and 10, and then spiked in the intial stages of puberty, concurrent with the rise in sex steroid production.

In the present investigation, inhibin B failed to significantly correlate with testosterone triggered by HCG ($p=0.649$).

In contrast with current results, **Kubini et al., (2000)** reported a substantial connection ($r = 0.84$; $P < 0.0001$) between basal inhibin B and the testosterone levels after HCG in 54 patients with 46 XY DSD.

Also, **Crofton et al., (2002)** in UK collected blood samples from 366 boys aged 0--18 years, they found that inhibin B had substantial positive correlation with testosterone ($P < 0.01$).

REFERENCES

1. **Ahmadifard, M., Kajbafzadeh, A., Panjeh-Shahi, S., Vand-Rajabpour, F., Ahmadi-Beni, R., Arshadi, H., Tabrizi, M. (2019).** Molecular investigation of mutations in androgen receptor and 5-alpha-reductase-2 genes in 46, XY Disorders of Sex Development with normal testicular development. Andrologia, 51(5), e13250.
2. **Ahmed, S., Cheng, A, & Hughes, I. (1999).** Assessment of the gonadotrophin–gonadal axis in androgen insensitivity syndrome. Archives of disease in childhood, 80(4), 324-329.
3. **Aksglaede, L., Sørensen, K., Boas, M., Mouritsen, A., Hagen, C., Jensen, R., Main, K. (2010).** Changes in anti-Mullerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. The Journal of Clinical Endocrinology & Metabolism, 95(12), 5357-5364.
4. **Andersson, A.M. & Skakkebæk, N.E. (2001).** Serum inhibin B levels during male childhood and puberty. Molecular and cellular endocrinology, 180(1-2), 103-107.
5. **Babbar, P. & Shah, A. (2019).** Disorders of Sexual Development. Comprehensive Care of the Transgender Patient E-Book, 25.
6. **Bennecke, E., Köhler, B., Röhle, R., Thyen, U., Gehrmann, K., Lee, P., Wiesemann, C. (2020).** Disorders or Differences of Sex Development? Views of Affected Individuals on DSD Terminology. The Journal of Sex Research, 1-10.
7. **Bergada, I., Bergada, C. & Campo, S. (2001).** Role of inhibins in childhood and puberty. Journal of Pediatric Endocrinology and Metabolism, 14(4), 343-354.
8. **Buonocore, F., Clifford-Mobley, O., King, T.F., Striglioni, N., Man, E., Suntharalingham, J.P., Rumsby, G. (2019).** Next-generation sequencing reveals novel genetic variants (SRY, DMRT1, NR5A1, DHH, DHX37) in adults with 46, XY DSD. Journal of the Endocrine Society, 3(12), 2341-2360.
9. **Crofton, P., Evans, A., Groome, N.P., Taylor, M., Holland, C. & Kelnar, C. (2002).** Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. Clinical endocrinology, 56(2), 215-221.
10. **Ekenze, S., Nwangwu, E., Amah, C., Agugua-Obianyo, N., Onuh, A. & Ajuzieogu, O. (2015).** Disorders of sex development in a developing country: perspectives and outcome of surgical management of 39 cases. Pediatric surgery international, 31(1), 93-99.
11. **Freire, A.V., Grinspon, R.P. & Rey, R.A. (2018).** Importance of serum testicular protein hormone measurement in the assessment of disorders of sex development. Sexual Development, 12(1-3), 30-40.
12. **Grinspon, R.P. & Rey, R.A. (2010).** Anti-müllerian hormone and sertoli cell function in paediatric male hypogonadism. Hormone Research in Paediatrics, 73(2), 81-92.
13. **Grinspon, R.P., Ropelato, M.G., Bedecarrás, P., Loreti, N., Ballerini, M. G., Gottlieb, S., Rey, R.A. (2012).** Gonadotrophin secretion pattern in anorchid boys from birth to pubertal age: pathophysiological aspects and diagnostic usefulness. Clinical endocrinology, 76(5), 698-705.

14. **Hafez, M., Abd El Dayem, S.M., El Mougy, F., Atef, A., Kandil, M. & Galal, A. (2014).** The role of anti-Mullerian and inhibin B hormones in the evaluation of 46, XY disorders of sex development. Journal of Pediatric Endocrinology and Metabolism, 27(9-10), 891-899.
15. **Josso, N., Rey, R.A., & Picard, J.Y. (2013).** Anti-müllerian hormone: a valuable addition to the toolbox of the pediatric endocrinologist. International journal of endocrinology, 2013.
16. **Kubini, K., Zachmann, M., Albers, N., Hiort, O., Bettendorf, M., Wölfle, J., Klingmüller, D. (2000).** Basal inhibin B and the testosterone response to human chorionic gonadotropin correlate in prepubertal boys. The Journal of Clinical Endocrinology & Metabolism, 85(1), 134-138.
17. **Lahlou, N., Bouvattier, C., Linglart, A., Rodrigue, D. & Teinturier, C. (2009).** The role of gonadal peptides in clinical investigation. Paper presented at the Annales de Biologie Clinique.
18. **Lasala, C., Carré-Eusèbe, D., Picard, J.Y., & Rey, R. (2004).** Subcellular and molecular mechanisms regulating anti-Müllerian hormone gene expression in mammalian and nonmammalian species. DNA and cell biology, 23(9), 572-585.
19. **Lee, M.M., Donahoe, P.K., Silverman, B.L., Hasegawa, T., Hasegawa, Y., Gustafson, M.L., MacLaughlin, D.T. (1997).** Measurements of serum Müllerian inhibiting substance in the evaluation of children with nonpalpable gonads. New England Journal of Medicine, 336(21), 1480-1486.
20. **Lee, P.A., Gollenberg, A. L., Hediger, M.L., Himes, J.H., Zhang, Z. & Louis, G.M.B. (2010).** Luteinizing hormone, testosterone and inhibin B levels in the peripubertal period and racial/ethnic differences among boys aged 6–11 years: analyses from NHANES III, 1988–1994. Clinical endocrinology, 73(6), 744-751.
21. **Lucas-Herald, A.K., Kyriakou, A., Alimussina, M., Guaragna-Filho, G., Diver, L.A., McGowan, R., Ahmed, S.F. (2020).** Serum anti-Müllerian hormone in the prediction of response to hCG stimulation in children with DSD. The Journal of Clinical Endocrinology & Metabolism, 105(5), 1608-1616.
22. **Majumdar, I. & Mazur, T. (2018).** Management of Infants Born with Disorders/Differences of Sex Development Pediatric Endocrinology (pp. 617-639): Springer.
23. **Mekkawy, M.K., Kamel, A.K., Dessouky, N., Elgharbawy, M. & Mazen, I. (2019).** Cytogenetic Spectrum of Ovotesticular Difference of Sex Development (OT DSD) among a Large Cohort of DSD Patients and Literature Review. Sexual Development, 13(5-6), 221-227.
24. **Ohnuma, K., Kaneko, H., Noguchi, J., Kikuchi, K., Ozawa, M. & Hasegawa, Y. (2007).**Production of inhibin A and inhibin B in boars: changes in testicular and circulating levels of dimeric inhibins and characterization of inhibin forms during testis growth. Domestic animal endocrinology, 33(4), 410-421.
25. **Raivio, T., Wikström, A.M., & Dunkel, L. (2007).** Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. European Journal of Endocrinology, 156(1), 105-111.
26. **Rey, R.A. & Grinspon, R. P. (2011).** Normal male sexual differentiation and aetiology of disorders of sex development. Best Practice & Research Clinical Endocrinology & Metabolism, 25(2), 221-238.
27. **Szarras-Czapnik, M., Gajewska, M., Ksiazyk, J., Janas, R. & Ginalska-Malinowska, M. (2006).** Anti-Müllerian hormone (AMH) measurements in the assessment of testicular function in prepubertal boys and in sexual differentiation disorders. Endokrynologia, diabetologia i choroby przemiany materii wieku rozwojowego: organ Polskiego Towarzystwa Endokrynologow Dzieciecych, 12(3), 195-199.
28. **Young, J., Chanson, P., Salenave, S., Noël, M., Brailly, S., sO'Flaherty, M.N., Rey, R. (2005).** Testicular anti-mullerian hormone secretion is stimulated by recombinant human FSH in patients with congenital hypogonadotropic hypogonadism. The Journal of Clinical Endocrinology & Metabolism, 90(2), 724-728.