0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(2):724–728

doi: 10.1210/jc.2004-0542

Testicular Anti-Müllerian Hormone Secretion Is Stimulated by Recombinant Human FSH in Patients with Congenital Hypogonadotropic Hypogonadism

Jacques Young, Philippe Chanson, Sylvie Salenave, Michèle Noël, Sylvie Brailly, Martín O'Flaherty, Gilbert Schaison, and Rodolfo Rey

Service d'Endocrinologie et des Maladies de la Reproduction (J.Y., P.C., S.S., G.S.) and Laboratoire d'Hormonologie (S.B.), Assistance Publique-Hôpitaux de Paris, Bicêtre Hospital and Faculté de Médecine Paris XI, 94270 Le Kremlin Bicêtre, France; Service de Biochimie-Hormonologie (M.N.), Hôpital Robert Debré, 75019 Paris, France; Facultad de Ciencias Biomédicas and Hospital Universitario (M.O.), Universidad Austral, B1629AJH Pilar, Buenos Aires, Argentina; and Centro de Investigaciones Endocrinológicas (R.R.), Hospital de Niños R. Gutiérrez, C1425EFD Buenos Aires, Argentina

Serum anti-Müllerian hormone (AMH), a prepubertal Sertoli cell marker, declines during puberty as an early sign of testicular testosterone (T) production. When T synthesis or action is impaired, serum AMH is abnormally high in the first months after birth and at puberty but normal between these two periods. We postulated that FSH might be responsible for AMH up-regulation in the absence of androgen inhibition.

To test this hypothesis, we administered recombinant human (rh) FSH to eight patients aged from 18–31 yr with untreated congenital hypogonadotropic hypogonadism. This situation is ideal to study the effect of FSH on AMH production because it avoids interference by endogenous gonadotropins and T. The patients received daily sc injections of 150 IU rhFSH for 1 month, followed in seven of them by a combined treatment of rhFSH plus human chorionic gonadotropin (hCG; 1500 UI im, twice a week) for 2 months. Gonadotropins, T, AMH, and inhibin B were measured in plasma before treatment every 10 d during rhFSH treatment and every month during combined rhFSH and hCG treatments.

All hormones were at prepubertal levels before treatment. Although LH and T did not vary, AMH and inhibin B levels gradually increased after 20 d of FSH administration. However, in contrast to rhFSH alone, the combined rhFSH plus hCG stimulation of the testis dramatically suppresses the secretion of AMH and induced a modest but significant reduction of circulating inhibin B levels.

We conclude that FSH stimulates AMH production in the testis when it is at a prepubertal stage. In addition, the decrease of serum AMH during combined rhFSH and hCG testicular stimulation is in agreement with the concept that during pubertal development and in adult life, the suppressive effect of LH-driven testicular androgens outweighs the stimulating effect of FSH on AMH production by Sertoli cells. Finally, the hCG-induced decrease in inhibin B suggests that in humans, as previously demonstrated in monkeys, testicular T is also able to inhibit inhibin B secretion. (*J Clin Endocrinol Metab* 90: 724–728, 2005)

THE ENDOCRINE FUNCTION of the testis shows developmental changes through fetal and postnatal life until adulthood. In the fetus and neonate, both Sertoli and Leydig cell populations actively secrete male hormones. Stimulated by human chorionic gonadotropin (hCG) in the first two trimesters of intrauterine life and by pituitary LH from the perinatal period through 3–6 months after birth, Leydig cells lying in the interstitial tissue of the testis secrete large amounts of testosterone (T). Thereafter, because of the physiologic fall in pituitary activity, Leydig cells are no longer seen in the gonads, and serum T levels remain low until the onset of puberty (1). Sertoli cells, the largely predominant cell population of the seminiferous tubules of the fetal and prepubertal testis, produce anti-Müllerian hormone (AMH) and inhibin B. In the male gonad, pituitary FSH receptors are found exclusively in Sertoli cells and enhance testicular inhibin B production (2–4); in turn, inhibin B exerts negative feedback control on pituitary FSH secretion in males (2, 5, 6). AMH, also known as Müllerian-inhibiting substance, is a Sertoli cell-specific product in males. AMH is secreted at high levels from early fetal life until the onset of puberty, when it is down-regulated by the increasing intratesticular concentration of T and the presence of meiotic germ cells (for review, see Ref. 7). Serum AMH is a useful marker of Sertoli cell activity in boys, and its decline during puberty is an early sign of local T activity and spermatogenic development in the testis (for review, see Refs. 8 and 9). In patients with impaired T synthesis, related to LH receptor mutations or steroidogenic protein defects or with end-organ insensitivity due to androgen receptor mutations, serum AMH levels are considerably elevated in the first months of postnatal life and after the onset of puberty but not between these two points (10). In prepubertal mice, FSH induces prepubertal Sertoli cell proliferation, thereby enlarging the AMH-secreting cell population, but it also activates AMH gene transcription through a novel pathway mediated by cyclic AMP (11, 12). Therefore, FSH might be the gonadotropin responsible for the increased AMH production seen in boys in whom the negative control normally exerted by androgens

First Published Online November 9, 2004

Abbreviations: AMH, Anti-Müllerian hormone; CHH, congenital hypogonadotropic hypogonadism; hCG, human chorionic gonadotropin; rh, recombinant human; T, testosterone.

is absent, *i.e.* boys with defective androgen synthesis or sensitivity. To test this hypothesis directly in humans, we administered recombinant human (rh) FSH to men with untreated congenital hypogonadotropic hypogonadism (CHH) and prepubertal-range T levels. This is an ideal situation to study the effect of FSH on testicular AMH production because it avoids the interference by endogenous gonadotropins and T observed in other physiological and pathological circumstances (13, 14). We also evaluated the hypothesis that suppressive effect of hCG-derived testicular T (13, 14) could outweigh the stimulating effect of FSH on AMH production by Sertoli cells. Finally, using this model, we had the opportunity to outline the relative role of FSH and hCG in regulating the testicular secretion of inhibin B in humans. Indeed, although FSH is recognized to stimulate testicular inhibin B secretion (4), the action of LH-hCG in man in this regard is less clear. We provide here evidence for the view that the gonadotropin control of testicular inhibin B in human involves opposing stimulatory and inhibitory actions of FSH and hCG, respectively.

Patients and Methods

Subjects

Eight previously untreated men, aged 18–31 yr, with idiopathic CHH (n = 4) or CHH due to Kallmann's syndrome (n = 4), were selected at hospital admission for diagnosis and choice of therapy. The diagnostic criteria for CHH were as follows: failure of spontaneous puberty, low testicular volume, and normal findings on cranial magnetic resonance imaging of the hypothalamic-pituitary region. Patients were considered to have Kallmann's syndrome if anosmia was also present. None of the patients had a history of cryptorchidism. All the patients had low plasma T levels and low gonadotropin plasma levels with a nonpulsatile LH profile. Basal and stimulated levels of cortisol, growth hormone, prolactin, and TSH in response to CRH, GHRH, and TRH were normal, as were basal thyroid hormone levels. None of the patients had previously received gonadotropin or androgen replacement therapy. All the patients were offered treatment consisting of FSH for 1 month, followed by FSH combined with hCG, to increase testicular size and virilization.

All subjects gave their informed consent to participate in the study, which was approved by the local human investigation committee.

Study design

At admission, a blood sample was drawn to determine baseline serum FSH, LH, T, AMH, and inhibin B levels before beginning gonadotropin administration. Each patient received rhFSH (GONAL-f, Laboratoires Serono, Aubonne, Switzerland), 150 IU/d sc at 0800 h for 1 month, followed in seven of them by a combined treatment of rhFSH (150 IU/d sc) plus hCG (Gonadotrophine-chorionique, Laboratoires Organon, Puteau, France) (1500 IU im, twice a week) for 2 months. Blood samples were drawn every 10 d at 1000 h during the rhFSH treatment and every month during the combined treatment periods, respectively. Testicular volume was measured using the Prader orchidometer (Pharmacia, St. Quentin-en-Yvelines, France).

Six of the patients studied had undergone an additional period of combined rhFSH (150 IU three times a week, sc) plus hCG (1500 IU im, twice a week) treatment of 12 months and submitted semen specimens for semen analyses once every 4 months. At four months of this combined treatment, semen analyses revealed in all the patients a complete azoospermia. Eight months later, sperm density rose from 0–3.1 ± 1.27 × 10^6 (range, 0.6–8.4 × 10^6).

Assays

Plasma LH and FSH were measured by immunofluorometric assay (Cis-Bio, Gif-sur-Yvette, France). The intra- and interassay coefficients of variation were 1.5 and 5.2% for LH and 2.6 and 4% for FSH, respectively (4). The detection limit was 0.15 IU/liter in both assays. Plasma T levels were measured by RIA after chromatography on a celite column, as previously described (15). The T detection limit was 0.17 nmol/liter, and the inter- and intraassay coefficients of variation were 6.0 and 5.8%. Plasma inhibin B levels were measured with a commercial ELISA (Serotec, Kidlington, Oxford, United Kingdom) with a detection limit of 10 pg/ml and inter- and intraassay coefficients of variation of 15 and 6%, respectively (4). Serum AMH levels were measured with an ELISA (AMH/Müllerian-inhibiting substance ELISA, Immunotech-Beckman, Marseilles, France), as previously described (16). The detection limit was 0.4 ng/ml, and inter- and intraassay coefficients of variation were 13.8 and 7.04%, respectively.

Statistical analyses

Hormone levels are reported as means and SEM values. We used one-way repeated measure ANOVA to assess the testicular response to rhFSH or to the combined rhFSH plus hCG treatment; the dependent variable was each of the testicular hormones studied. Appropriate *post hoc* pair-wise comparisons were performed. $P < 0.05$ was considered to denote statistical significance. All statistical analyses were run on STATA 8.0 software.

Results

The characteristics of the subjects at admission are described in Table 1. All the patients had prepubertal levels of T and gonadotropins. After rhFSH administration, circulating FSH levels increased (ANOVA F = 25.76, $P < 0.001$), but T and LH levels remained at prepubertal levels throughout the study (ANOVA F = 1.45 and 0.44 respectively, not significant) (Table 2). Baseline inhibin B levels were below normal for Tanner stage, but, as we and others have previously reported (2–4), they increased gradually from d 20 of treatment with rhFSH (ANOVA F = 87.9, $P < 0.001$) (Table 2) in all the patients (Fig. 1).

TABLE 1. Clinical characteristics and serum hormone levels at diagnosis in men with CHH

Patient	Age (yr)	Diagnosis	Testicular volume (ml)	LH (IU/liter)	FSH (IU/liter)	Testosterone (nmol/liter)	Inhibin B (pg/ml)	AMH (pmol/liter)
1	18	Kallmann's syndrome	2	0.4	0.3	0.7	37	450
2	19	Kallmann's syndrome	2	0.3	0.3	1.7	38	193
3	20	Kallmann's syndrome	2	0.1	0.3	1.0	32	157
4	22	Kallmann's syndrome	2	0.2	0.4	1.7	34	371
5	19	Idiopathic	1	0.1	0.1	0.5	26	286
6	26	Idiopathic	6	0.9	1.2	2.4	74	471
7	29	Idiopathic	4	0.6	0.8	1.7	42	236
8	31	Idiopathic	4	0.5	0.7	1.4	46	550
		Normal range						
		Prepubertal	2–4	0.1–0.9	0.2–1.8	0.15–1.8	36–212	360–640
		Adult	12–25	4–8	4–7	12–28	105–360	<80

TABLE 2. Serum hormone levels (mean ± SEM) in men with CHH during 30 d of rhFSH administration

	d 0	d 10	d 20	d 30
LH (IU/liter)	0.39 ± 0.10	0.44 ± 0.12	0.47 ± 0.14	0.41 ± 0.09
Testosterone (nmol/liter)	1.37 ± 0.22	1.17 ± 0.18	1.30 ± 0.16	1.04 ± 0.22
FSH (IU/liter)	0.34 ± 0.07	11.38 ± 1.05[a]	13.00 ± 0.82[a]	14.63 ± 0.91[a]
AMH (pmol/liter)	339 ± 51	345 ± 44	459 ± 46[a,b]	554 ± 58[a,b,c]
Inhibin B (pg/ml)	40.3 ± 5.6	44.5 ± 6.0	66.8 ± 5.1[a,b]	92.0 ± 5.3[a,b,c]

See *Patients and Methods*.
[a] $P < 0.001$ *vs.* d 0; [b] $P < 0.001$ *vs.* d 10; [c] $P < 0.01$ *vs.* d 20.

Baseline serum AMH levels were high for chronological age but not for Tanner stage (Table 1). Furthermore, in two patients with Kallmann's syndrome and two patients with idiopathic hypogonadotropic hypogonadism, AMH levels were clearly below those expected in prepubertal males. Although no significant difference was observed after 10 d of treatment with rhFSH, serum AMH increased gradually at 20 and 30 d in all the patients (ANOVA F = 96.72, $P < 0.001$) (Table 2 and Fig. 1).

In the seven CHH patients who had undergone the combined rhFSH plus hCG treatment, after 1 month T increased from 1.1 ± 0.18 to 10.5 ± 0.9 nmol/liter, and serum AMH levels decreased in all the patients (Fig. 2) from 552 ± 65 to 260 ± 42 pmol/liter ($P < 0.02$). A further decrease of serum AMH (to 52 ± 12 pmol/liter) was observed when the combined therapy was continued for 2 months (Fig. 2). After this combined therapy, mean serum FSH levels (13.8 ± 0.9 and 14.2 ± 1.2 IU/liter at 1 and 2 months, respectively) were not significantly different from those observed after 1 month of rhFSH alone (14.6 ± 0.91 IU/liter).

In addition, a modest but significant decrease of serum inhibin B levels was observed in the seven CHH patients after 2 months of combined rhFSH plus hCG treatment (from 92 ± 6.0 to 75 ± 6.6 pg/ml; $P < 0.02$) when compared with levels achieved during rhFSH treatment alone (Fig. 3). However, at that time, mean serum inhibin B levels remained significantly higher than those observed at baseline (75 ± 6.6 *vs.* 41 ± 5.2 pg/ml; $P < 0.02$).

Discussion

To test whether immature Sertoli cells were capable of responding to FSH stimulation with an enhanced production of AMH resulting in an elevation of serum levels in humans, we treated eight patients with a congenital complete deficiency of gonadotropin secretion exclusively with rhFSH for 1 month. Our results clearly demonstrate that FSH induces an increase of serum AMH levels. This might seem controversial with previous findings in normal boys, in whom testicular AMH production decreases when FSH levels increase at the onset of puberty (8, 9). However, we previously showed that the dramatic inhibition of AMH expression at puberty results from the increasing concentration of T within the testis, acting via the androgen receptor expressed by pubertal and adult Sertoli cells (11, 13). In the present study, we demonstrated that the suppressive effect of hCG-derived testicular T (13) can outweigh the stimulating effect of FSH on AMH production by Sertoli cells. Therefore, these data are consistent with the hypothesis that in humans during pubertal development and in adult life, the negative effect of LH-derived androgens is clearly predominant over the stimulating effect of FSH on AMH production by Sertoli cells. On the other hand, it was intriguing that in patients in whom T synthesis was impaired or target cells were not sensitive to androgens (androgen insensitivity syndromes), AMH levels were extremely high in coincidence with the activation of gonadotropin secretion, *i.e.* in the first months after birth and at the age of puberty, but not during the rest of childhood when gonadotropin levels are physiologically low (10). Our present results are in line with the hypothesis that FSH is responsible for the abnormal elevation of serum AMH observed in those patients. In fact, here we show that, in patients lacking androgen activity within the testis, FSH is capable of increasing AMH secretion by Sertoli cells (Fig. 1).

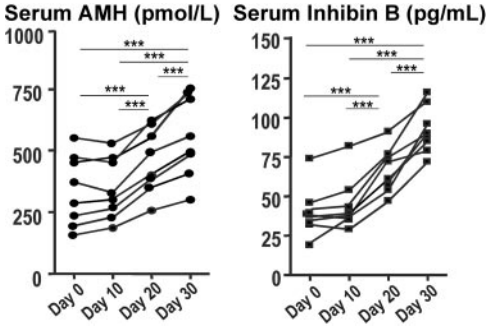

FIG. 1. Individual serum AMH and inhibin B levels in eight previously untreated men with CHH who received a daily injection of rhFSH (150 IU sc) for 30 d. d 0, Sample taken immediately before the first FSH injection. ***, $P < 0.001$.

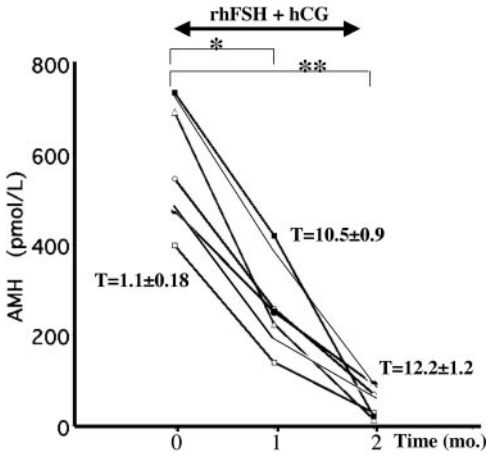

FIG. 2. Individual serum AMH levels in seven men with hypogonadotropic hypogonadism previously treated with rhFSH (see Fig. 1 and *Patients and Methods*). Effect of the simultaneous administration of rhFSH and hCG. *, $P < 0.02$; **, $P < 0.01$. T, Mean plasma T levels (nanomoles per liter ± SEM).

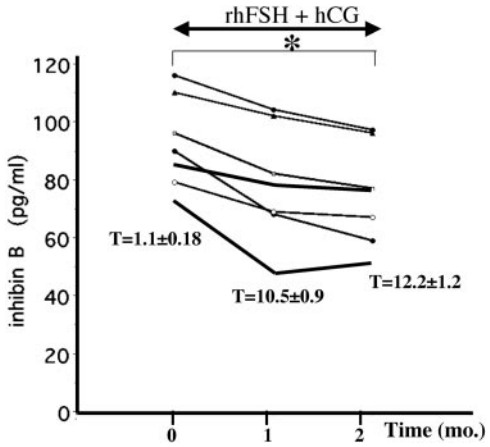

FIG. 3. Individual serum inhibin B levels in seven men with hypogonadotropic hypogonadism previously treated with rhFSH (see Fig. 1 and *Patients and Methods*). Effect of the simultaneous administration of rhFSH and hCG. *, $P < 0.02$. T, mean plasma T levels (nanomoles per liter ± SEM).

The effect of FSH on testicular AMH production might be due to a direct effect on AMH expression in each individual Sertoli cell, a proliferative effect on Sertoli cells, or both. Clinical tools (*e.g.* testis palpation, ultrasonography) are not sensitive enough to assess the small absolute increase in gonadal volume that might derive from Sertoli cell proliferation. Therefore, we cannot answer this question with the present data. However, experimental studies in mice have clearly shown that FSH increases testicular AMH production because of both Sertoli cell proliferation and enhancement of AMH expression in individual Sertoli cells (12).

The evaluation of endocrine testicular activity in childhood has classically relied on the determination of serum T, which needs hCG stimulation after the 6th month of life until the onset of puberty. Although largely accepted as a test of male gonadal function, androgen assay only reflects the steroidogenic activity of interstitial Leydig cells. However, the prepubertal testis is mainly composed of Sertoli cells, which represent more than 75% of gonadal mass (17, 18) and are unquestionably active (1, 19). AMH, like inhibin B, is a useful marker of testicular function in the prepubertal male. It can be assayed, without the need for stimulation, in an attempt to determine the existence of testicular tissue in patients with nonpalpable gonads. Here, we show that AMH determination may also be used to explore the functional reserve in response to FSH. Because the spermatogenic potential of the testis is dependent on Sertoli cell function (20), which, in turn, is regulated by FSH (21–23), basal and FSH-stimulated levels of AMH, like those of inhibin B (24, 25), might become a useful predictive marker of the spermatogenic response to gonadotropic treatment in patients with hypogonadotropic hypogonadism. However, further studies, with an adequate design to approach a problem of prognosis, are necessary to test this hypothesis.

We found that rhFSH administration took between 10 and 20 d to induce a significant elevation of serum AMH and inhibin B levels. Although this might seem long, the same lag period is observed after an hCG test in previously untreated patients with CHH. In this latter case too, the amplitude of the response is small, and longer stimulation is needed to obtain significant steroidogenic activity. In contrast, inhibin B levels rise more rapidly in response to rhFSH stimulation in patients with acquired hypogonadotropic hypogonadism who were exposed to gonadotropins in fetal and neonatal life (4) and also in normal males (2).

In contrast to rhFSH alone, combined rhFSH plus hCG stimulation of the testis appears to suppress secretion of inhibin B. This result is in agreement with data obtained with juvenile monkeys, in which LH infusion for 11 d reduced circulating inhibin B levels and curtailed the stimulatory action of FSH on inhibin B secretion (26). A plausible explanation for the inhibitory action of hCG on testicular inhibin B secretion is that it is indirect and mediated by paracrine action of increased T production from the Leydig cells (14). This view is supported by the finding that the time course of the hCG-induced suppression of inhibin B is inversely related to the increase of T and is in line with the finding that the implantation of T-filled capsules in hypogonadotropic adult monkeys resulted in suppression in inhibin B secretion by the testis (26). Lastly, the decrease in inhibin B levels despite the concomitant administration of rhFSH, which allowed the maintenance of the serum FSH levels attained with rhFSH alone, excluded an indirect effect of hCG, mediated by the inhibition of pituitary FSH secretion (27).

In summary, we demonstrate that in the absence of the androgen-inhibitory effect, FSH is able to enhance testicular AMH secretion in man. However, the fact that the hCG-derived testicular androgens could outweigh the stimulating effect of FSH indicate that intratesticular T is the most potent inhibitor of postnatal AMH secretion and explain the decrease in AMH levels at the onset of puberty despite the increase in FSH secretion.

Acknowledgments

Received March 24, 2004. Accepted November 3, 2004.

Address all correspondence and requests for reprints to: Jacques Young, M.D., Ph.D., Service d'Endocrinologie et des Maladies de la Reproduction, Hôpital Bicêtre, 94270 Le Kremlin Bicêtre cedex, France. E-mail: jacques.young@bct.ap-hop-paris.fr.

References

1. **Chemes HE** 2001 Infancy is not a quiescent period of testicular development. Int J Androl 24:2–7
2. **Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS, Bremner WJ** 1996 Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. J Clin Endocrinol Metab 81:3341–3345
3. **Raivio T, Toppari J, Perheentupa A, McNeilly AS, Dunkel L** 1997 Treatment of prepubertal gonadotrophin-deficient boys with recombinant human follicle-stimulating hormone. Lancet 350:263–264
4. **Young J, Couzinet B, Chanson P, Brailly S, Loumaye E, Schaison G** 2000 Effects of human recombinant luteinizing hormone and follicle-stimulating hormone in patients with acquired hypogonadotropic hypogonadism: study of Sertoli and Leydig cell secretions and interactions. J Clin Endocrinol Metab 85:3239–3244
5. **Anderson RA, Wallace EM, Groome NP, Bellis AJ, Wu FC** 1997 Physiological relationships between inhibin B, follicle stimulating hormone secretion and spermatogenesis in normal men and response to gonadotrophin suppression by exogenous testosterone. Hum Reprod 12:746–751
6. **Bergadá I, Rojas G, Ropelato G, Ayuso S, Bergadá C, Campo S** 1999 Sexual dimorphism in circulating monomeric and dimeric inhibins in normal boys and girls from birth to puberty. Clin Endocrinol (Oxf) 51:455–460
7. **Rey R** 1998 Endocrine, paracrine and cellular regulation of postnatal anti-Müllerian hormone secretion by Sertoli cells. Trends Endocrinol Metab 9:271–276

8. **Lane AH, Lee MM** 1999 Clinical applications of Mullerian inhibiting substance in patients with gonadal disorders. Endocrinologist 9:208–215
9. **Rey R** 2000 Assessment of seminiferous tubule function (anti-Müllerian hormone). Baillieres Best Pract Res Clin Endocrinol Metab 14:399–408
10. **Rey R, Mebarki F, Forest MG, Mowszowicz I, Cate RL, Morel Y, Chaussain JL, Josso N** 1994 Anti-müllerian hormone in children with androgen insensitivity. J Clin Endocrinol Metab 79:960–964
11. **Al-Attar L, Noël K, Dutertre M, Belville C, Forest MG, Burgoyne PS, Josso N, Rey R** 1997 Hormonal and cellular regulation of Sertoli cell anti-Müllerian hormone production in the postnatal mouse. J Clin Invest 100:1335–1343
12. **Lukas-Croisier C, Lasala C, Nicaud J, Bedecarrás P, Kumar TR, Dutertre M, Matzuk MM, Picard JY, Josso N, Rey R** 2003 Follicle-stimulating hormone increases testicular anti-Müllerian hormone (AMH) production through Sertoli cell proliferation and a nonclassical cyclic adenosine 5′-monophosphate-mediated activation of the AMH gene. Mol Endocrinol 17:550–561
13. **Young J, Rey R, Couzinet B, Chanson P, Josso N, Schaison G** 1999 Anti-müllerian hormone in patients with hypogonadotropic hypogonadism. J Clin Endocrinol Metab 84:2696–2699
14. **Young J, Rey R, Schaison G, Chanson P** 2003 Hypogonadotropic hypogonadism as a model of post-natal testicular anti-müllerian hormone secretion in humans. Mol Cell Endocrinol 211:51–54
15. **Young J, Couzinet B, Pholsena M, Nahoul K, Labrie F, Schaison G** 1994 Plasma 3 β-hydroxy-delta 5-steroids in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 78:299–304
16. **Rey RA, Belville C, Nihoul-Fékété C, Michel-Calemard L, Forest MG, Lahlou N, Jaubert F, Mowszowicz I, David M, Saka N, Bouvattier C, Bertrand AM, Lecointre C, Soskin S, Cabrol S, Crosnier H, Léger J, Lortat-Jacob S, Nicolino M, Rabl W, Toledo SP, Bas F, Gompel A, Czernichow P, Josso N** 1999 Evaluation of gonadal function in 107 intersex patients by means of serum antimüllerian hormone measurement. J Clin Endocrinol Metab 84:627–631
17. **Nistal M, Abaurrea MA, Paniagua R** 1982 Morphological and histometric study on the human Sertoli cell from birth to the onset of puberty. J Anat 134:351–363
18. **Muller J, Skakkebæk NE** 1983 Quantification of germ cells and seminiferous tubules by stereological examination of testicles from 50 boys who suffered from sudden death. Int J Androl 6:143–156
19. **Rey R** 1999 The prepubertal testis: a quiescent or a silently active organ? Histol Histopathol 14:991–1000
20. **Orth JM, Gunsalus GL, Lamperti AA** 1988 Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinology 122:787–794
21. **Orth JM** 1984 The role of follicle-stimulating hormone in controlling Sertoli cell proliferation in testes of fetal rats. Endocrinology 115:1248–1255
22. **Kumar TR, Palapattu G, Wang P, Woodruff TK, Boime I, Byrne MC, Matzuk MM** 1999 Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. Mol Endocrinol 13:851–865
23. **Wreford NG, Kumar TR, Matzuk MM, de Kretser DM** 2001 Analysis of the testicular phenotype of the follicle-stimulating hormone β-subunit knockout and the activin type II receptor knockout mice by stereological analysis. Endocrinology 142:2916–2920
24. **Nachtigall LB, Boepple PA, Seminara SB, Khoury RH, Sluss PM, Lecain AE, Crowley WFJ** 1996 Inhibin B secretion in males with gonadotropin-releasing hormone (GnRH) deficiency before and during long-term GnRH replacement: relationship to spontaneous puberty, testicular volume, and prior treatment: a clinical research center study. J Clin Endocrinol Metab 81:3520–3525
25. **Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT, Crowley WF** 2002 The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 87:152–160
26. **Ramaswamy S, Marshall GR, Pohl CR, Friedmann RL, Plant T** 2003 Inhibitory and stimulatory regulation of testicular inhibin B secretion by luteinizing hormone and follicle-stimulating hormone, respectively, in the rhesus monkey (*Macaca mulatta*). Endocrinology 144:1175–1185
27. **Lahlou N, Fennoy I, Carel JC, Roger M** 2004 Inhibin B and antimullerian hormone, but not testosterone levels, are normal in infants with nonmosaic Klinefelter syndrome. J Clin Endocrinol Metab 89:1864–1868

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.