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ORIGINAL ARTICLE *Reproductive endocrinology*

Individual serum levels of anti-Müllerian hormone in healthy girls persist through childhood and adolescence: a longitudinal cohort study

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BACKGROUND: In adult women, the circulating level of anti-Müllerian hormone (AMH) is a novel marker of ovarian function, as it reflects the number of remaining ovarian follicles. Therefore, AMH has gained widespread attention in fertility clinics, and a low AMH is believed to predict impaired fertility and imminent menopause. However, the natural course of circulating AMH levels during female childhood and adolescence is not known.

METHODS: Serum levels of AMH and FSH were measured in girls participating in The COPENHAGEN Puberty Study. Longitudinal part: 85 healthy girls and adolescents were examined, and blood samples were drawn every 6 months for an average of 3 years: median (range) number of samples per girl was 6 (2–10), age at baseline was 9.2 (5.9–12.9) years. Cross-sectional part: 224 prepubertal girls (age 8.3, 5.6–11.7 years) were examined and each girl had one blood sample drawn.

RESULTS: The individual mean AMH levels in girls followed longitudinally ranged from 5 to 54 pmol/l (median 18 pmol/l). The mean intra-individual coefficient of variation of AMH was 22% (range 0–54%). Overall, each girl maintained her AMH level throughout childhood and adolescence although minor, but significant, changes occurred during pubertal transition. In prepubertal girls, AMH was negatively correlated with FSH ($r = -0.31$, $P < 0.001$). Twelve per cent (10/85) had mean AMH below a cut-off value of 8 pmol/l, indicating that the interpretation of low AMH as a marker of approaching menopause may not apply to pre- and peri-pubertal girls.

CONCLUSIONS: Circulating AMH exhibits only minor fluctuations during childhood and adolescence, and a random AMH measurement seems representative for a given girl. The negative AMH–FSH correlation in prepubertal girls supports the notion that AMH is a quantitative marker of ovarian follicles even in young girls.

Key words: ovary / ovarian reserve / AMH / MIS / female reproduction

Introduction

Primordial follicles in the human ovary are only formed during fetal life, and the potential of female reproductive capacity is therefore already established at birth (Baker, 1963). Even during childhood, the number of ovarian follicles declines with increasing age. When the follicle number falls below a critical threshold of a few thousand, the menstrual cycle becomes irregular as menopause approaches (Richardson *et al.*, 1987).

Anti-Müllerian hormone (AMH) is produced by granulosa cells surrounding follicles that have undergone recruitment from the primordial follicle pool but have not been selected for dominance (pre-antral and early antral follicles) (Andersen *et al.*, 2010). In adult women, serum AMH level is considered to be a predictor of the follicle reserve.

High AMH levels are associated with high antral follicle count (de Vet et al., 2002), and with a high number of resting primordial follicles (Hansen et al., 2011). Unlike FSH, LH and estradiol, AMH appears to be relatively stable through the menstrual cycle (Hehenkamp et al., 2006), and levels are not affected by hormonal contraceptive treatment (Streuli et al., 2008). Thus, the evaluation of AMH is straight forward and has gained widespread attention and clinical use among gynaecologists and fertility doctors.

Patients with Turner syndrome (TS) experience primary ovarian insufficiency due to highly accelerated loss of follicles (Singh and Carr, 1966). Recently, we reported that a random serum AMH level below a cut-off value of 8 pmol/l was a highly specific and sensitive marker of ovarian failure in young TS patients. In the same study, we reported a stable but wide reference range of circulating AMH levels during childhood and adolescence in healthy girls (4.5–62.0 pmol/l) (Hagen et al., 2010a). The observed high prevalence of low AMH (<8 pmol/l) among otherwise healthy girls caused concern. However, due to the cross-sectional design of that study, it was not possible to discern whether the wide range of individual AMH levels reflected large inter-individual differences or, alternatively, intra-individual fluctuations.

FSH is measurable in all healthy prepubertal girls. Levels range from 0.3 to 6.8 IU/l (Sehested et al., 2000). It is not known if inter-individual FSH variation in healthy girls reflects different levels of ovarian activity.

We hypothesized that AMH is a marker of ovarian follicles even in young girls. By longitudinal evaluation of circulating AMH levels in a large contemporary group of healthy Danish girls, we expected to find slightly declining individual AMH levels during childhood and adolescence. A negative correlation of AMH and FSH levels in prepubertal girls would suggest an individual pituitary-ovarian set-point. This would support the notion that AMH is a quantitative marker of ovarian function even in young girls.

Materials and Methods

The COPENHAGEN Puberty Study is a combined cross-sectional and ongoing longitudinal population-based cohort study of healthy Danish children and adolescents. The primary purpose of conducting the study was to establish the time of pubertal onset in a contemporary cohort of Danish children (Aksglaede et al., 2009; Sorensen et al., 2010). Predefined secondary outcomes were to establish normative data of anthropometry and hormone levels during childhood and adolescence. All pupils ($n = 6203$) at 10 randomly selected schools within a radius of 10 km in the Copenhagen area were invited to participate in the cross-sectional study (2006–2008). All schools were situated in areas of Copenhagen traditionally representing the social middle class. The overall participation rate was 30%, ranging from 19 to 40%. Generally, the participation rates were highest in the youngest age groups. The participation rate was lowest in a college with adolescents from 14 to 18 years of age. All participants at two schools with high participation rates were invited to continue in the ongoing longitudinal follow-up study. Blood samples were drawn, and a thorough clinical examination was performed in all participating girls at every visit, including staging of breast development by palpation (B1–B5), according to Tanner's classification (Tanner, 1962). The pubertal onset was defined as having Tanner's breast stage 2 (B2) or more, as previously described in details (Aksglaede et al., 2009).

Longitudinal study population

To evaluate the inter- and intra-individual variation of hormone levels in serum during childhood, 108 girls and adolescents were examined every 6 months. The girls had no history of gynaecological diseases. Twenty-three girls were excluded from the present data set because one or both parents originated from a non-European country ($n = 11$), because none or only one AMH value was available ($n = 11$), and due to previous cytostatic treatment ($n = 1$). The remaining 85 healthy girls were included in the present study. They were followed for an average of 3 years (range: 0.5–4 years). Mean (range) age at baseline was 9.2 (5.9–12.9) years. AMH and FSH were evaluated in a total of 504 samples (median 6, range 2–10 per girl) and 502 (6, 1–10), respectively.

The age of pubertal onset was approximated using the date exactly between two visits where the girl advanced from B1 to B2 (or more). Thirty-nine girls entered puberty and 9 had menarche during the follow-up period.

Cross-sectional study population

In order to establish normative data of reproductive hormone levels during childhood and adolescence, blood samples were drawn from 995 healthy girls and adolescents. To evaluate the correlation between AMH and FSH levels in prepubertal girls, we included all 230 girls (age 5.6–11.7 years) without breast development (Tanner stage B1 on both sides) participating in the cross-sectional cohort. Six girls were excluded from the present study because one or both parents originated from a non-European country. Other aspects have previously been published (Hagen et al., 2010a).

Laboratory analyses

All blood samples were drawn between 8:00 a.m. and 1:00 p.m. from an antecubital vein, clotted, centrifuged and serum was stored at −20°C until hormone analyses were performed. Blood samples were analysed after a maximum of 4 years of storage in the freezer at −20°C. All samples were analysed in the same laboratory blinded for the technician for age and pubertal stage.

Reproductive hormone assays

Serum AMH levels were determined using the Beckman Coulter enzyme immunometric assay (Immunotech, Beckman Coulter Ltd., Marseilles, France) with a detection limit of 2.0 pmol/l. The intra-assay coefficients of variation (CVs) were <7.8 and 5.4% at 13 and 123 pmol/l, respectively. On the basis of results from the first 154 assays (corresponding to three batches), the inter-assay CVs were <11.3 and 9.2% at 18 and 99 pmol/l, respectively. In the following batches, several of the low and medium controls were above +2 SD. According to standard practice, all samples were adjusted using a batch-specific correction factor. Adjustments were blinded for age and stage of pubertal development. After adjustment of internal controls, the inter-assay CVs were 10.8 and 9.2% at 18 and 99 pmol/l, respectively.

Our AMH results were compared with those of other studies using another assay and other units. At present, two different AMH assays are commercially available: Immunotech, Beckman Coulter (BC) and Diagnostic System Laboratories (DSL). The recorded value of a specific serum AMH level is higher when measured on BC compared with DSL, and the detection limit of DSL is approximately five times lower than BC. To compare levels measured on different assays, the following conversion was used: AMH(BC) pmol/l = AMH(DSL) μg/l × 2.0 × 7.14 pmol/μg (Hehenkamp et al., 2006).

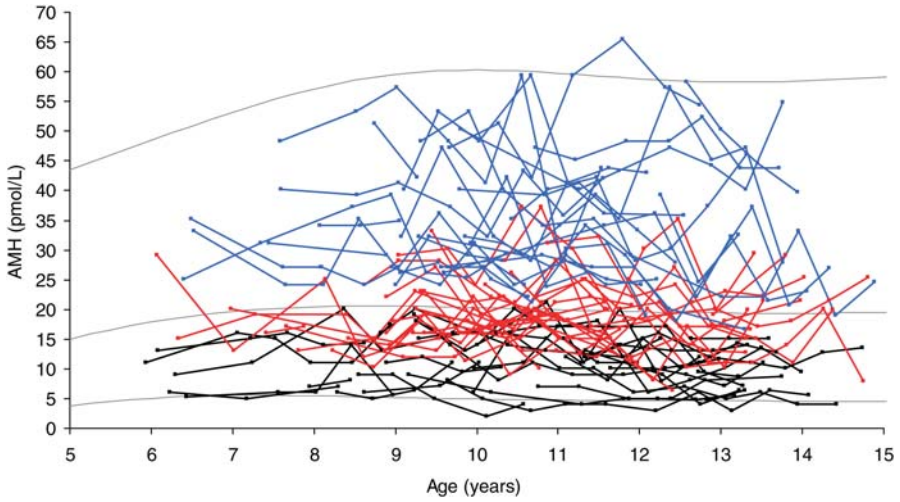

Figure 1 Longitudinal serum levels of AMH (pmol/l) in 85 healthy girls and adolescents as a function of age. Total number of samples (median; range of samples per girl): 504 (6; 2–10). The girls were grouped according to mean AMH level. Blue lines: 28 girls with the highest tertile of AMH; Red lines: 29 girls with the medium tertile of AMH; Black lines: 28 girls with the lowest tertile of AMH. Thin grey lines indicate the AMH reference range: median, 2.5th and 97.5th percentile.

Serum FSH was measured by time-resolved immunofluorometric assays (Delfia; PerkinElmer, Boston, MA, USA) with detection limits of 0.06 IU/l. Intra- and inter-assay CV were <5%.

Statistical analyses

To evaluate the individual fluctuation of AMH levels according to age, we compared intra-individual CV with the AMH inter-assay CV (CV% = SD/mean × 100). To evaluate the progress of AMH levels as a function of time from pubertal onset, we used a variance component model allowing each girl to have her own general AMH level. The time from B2 (numeric variable) was grouped into a categorized variable (i.e. $-0.5 \leq 0$ year <0.5; $0.5 \leq 1$ year <1.5; etc.). In case of multiple AMH values per girl in a given category, the mean AMH was used. To compensate for a skewed distribution of AMH, we transformed AMH values with the natural logarithm before analysis.

To enable visual evaluation of the association between longitudinal AMH and FSH levels as a function of age, longitudinal FSH levels were grouped in tertiles, according to the mean AMH level of the girl.

To assess the correlations between AMH and FSH levels in prepubertal girls (cross-sectional study), Spearman's correlation was used.

Ethical considerations

The Copenhagen Puberty Study (ClinicalTrials.gov ID: NCT01411527) was approved by the local ethical committee (KF 01 282214 and V200.1996/90) and the Danish Data Protection Agency (2010-41-5042). All children and parents received written information, and they were invited to an information meeting. All participants and their parents gave informed consent.

Results

Serum AMH was detectable (>2 pmol/l) in all samples. The individual mean AMH ranged from 5 to 54 pmol/l (median: 18 pmol/l). Overall, each girl maintained her AMH level throughout childhood and adolescence (Fig. 1). The mean intra-individual CV of AMH was 22.0% (range 0.1–53.7%). A total of 10 girls (12%) had a mean AMH level below a cut-off value of 8 pmol/l. Six of these 10 girls demonstrated ongoing pubertal development, whereas the remaining four did not enter puberty during follow-up. In the prepubertal samples from the girls with AMH <8 pmol/l, FSH levels ranged from 0.8 to 4.9 IU/l.

From 3 years prior to pubertal onset until 4 years after pubertal onset, individual AMH levels did not change significantly (from an average of 20–17 pmol/l, $P = 0.082$). During this period, AMH levels increased by 17% from 3 years prior to time of pubertal onset until start of puberty (from an average of 20–24 pmol/l, $P = 0.023$). After pubertal onset, AMH decreased 30% during the first 2 years (from an average of 24–17 pmol/l, $P < 0.001$). Subsequently, AMH levels were constant during the last 2 years of follow-up.

We found no correlation between individual AMH level (the mean level of AMH prior to pubertal onset) and age at entering puberty (Spearman: $r = 0.14$, $P = 0.39$).

Longitudinal FSH levels are shown according to AMH tertile groups as a function of chronological age (Fig. 2). Individual AMH levels were negatively associated with FSH levels; girls in the high AMH tertile (blue lines) having clearly lower FSH levels compared with girls in the low AMH tertile (black lines). The association was confirmed by a significant negative correlation between AMH and FSH serum levels in 224 prepubertal girls from the cross-sectional cohort (Spearman: $r = -0.31$, $P < 0.001$).

Discussion

To our knowledge, longitudinal AMH has not previously been evaluated in girls and adolescents, and this is the most comprehensive

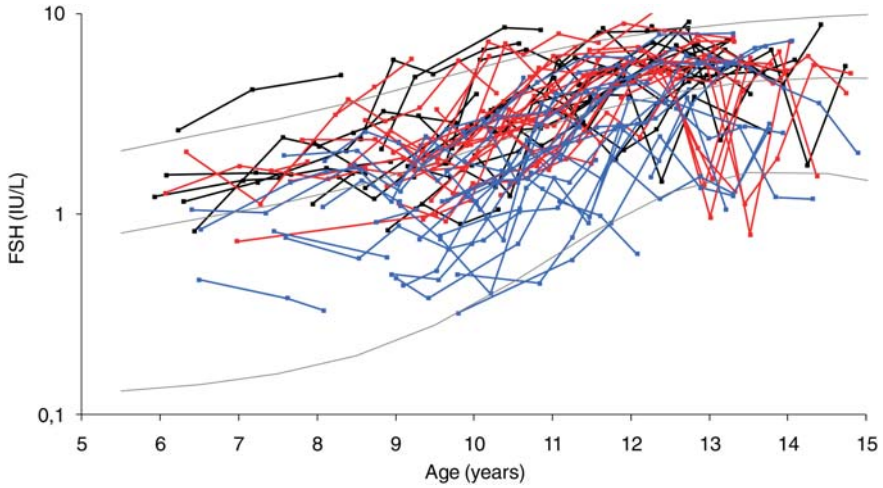

Figure 2 Longitudinal serum levels of FSH (IU/l) in 85 healthy girls and adolescents as a function of age. Total number of samples (median; range of samples per girl): 502 (6; 1–10). The girls were grouped according to mean AMH level. Blue lines: 28 girls with the highest tertile of AMH; Red lines: 29 girls with the medium tertile of AMH; Black lines: 28 girls with the lowest tertile of AMH. Thin grey lines indicate the FSH reference range: median, 2.5th and 97.5th percentile.

study of circulating AMH levels evaluated longitudinally in healthy females at all ages. We found that circulating AMH showed only minor fluctuations during childhood and adolescence. Thus, a random AMH measurement seems representative for a given girl.

Furthermore, AMH was negatively correlated with FSH prior to pubertal onset. Thus, each girl seems to have a prepubertal set-point of ovarian-pituitary activity, which may be determined by the number of ovarian follicles. Follicles large enough to produce AMH are present in prepubertal girls (Holm *et al.*, 1995). Our findings support the notion of a random AMH value being a marker of pre-antral and early antral follicles even in girls. Inter-individual variation of AMH levels may also be affected by the presence of polycystic ovarian syndrome and insulin levels (Codner *et al.*, 2011; Hart *et al.*, 2010). Unfortunately, such data are not available from our present study. FSH may indirectly affect circulating AMH levels by inducing follicle growth, which reduces the number of AMH-producing follicles (La Marca *et al.*, 2004). We speculate that the observed minor changes of AMH at pubertal onset are caused by redistribution of the follicle pool under the influence of the pubertal FSH surge.

Our finding of an individual prepubertal pituitary-ovarian set-point suggests that FSH is a marker of ovarian function in healthy girls during childhood. However, central inhibition of gonadotrophin secretion during mid-childhood makes elevated FSH an insensitive marker of ovarian failure in young patients with ovarian dysgenesis (Hagen *et al.*, 2010b).

In mammals, the onset of puberty is believed to be centrally regulated by pulsatile hypothalamic secretion of GnRH, which is largely unaffected by ovarian function (Pohl *et al.*, 1995). In patients with TS, the age of the pubertal gonadotrophin surge does not depend on the remaining ovarian function (Hagen *et al.*, 2010b). Thus, we expected to find that time of pubertal onset was not correlated with the prepubertal AMH level.

Serum AMH concentration has gained widespread attention as a marker of ovarian reserve in adult women, and AMH measurement in a single spot sample is now clinically used among fertility doctors worldwide. Longitudinal studies of AMH in adult women suggest that individual AMH levels decline over time, reflecting the continuous loss of follicles with age (van Rooij *et al.*, 2005). Furthermore, low AMH in healthy adults predicts early time of menopause (Broer *et al.*, 2011; Tehrani *et al.*, 2011). Using the exact same AMH assay as reported in our present study, we have previously found that an AMH level <8 pmol/l was a specific and sensitive marker of premature ovarian failure in young patients with TS (Hagen *et al.*, 2010a). Others have found that 0.56 μg/l (corresponding to 8 pmol/l) equals the median AMH level in 44-year-old women (van Disseldorp *et al.*, 2008). Although circulating AMH levels may reflect different physiological conditions in this cohort compared with adult women or TS patients, we are concerned by the high prevalence (12%) of apparently healthy girls maintaining AMH levels <8 pmol/l. In the 1980's, the incidence of primary ovarian insufficiency was 1% (Coulam *et al.*, 1986). At this time, there is no fertility outcome on this study population available. Continuous longitudinal follow up is essential to evaluate if low AMH is predictive of reduced fertility and premature ovarian insufficiency in this cohort. Theoretically, low pre- and peripubertal AMH levels may have a different clinical implication than later in life, and the interpretation of low AMH as a marker of approaching menopause may not apply to pre- and peri-pubertal girls. One study suggests that AMH declines rapidly 5 years prior to time of menopause (Sowers *et al.*, 2008). Longitudinal tracking of the steep premenopausal decline of AMH may be a more sensitive marker of approaching menopause in healthy females compared with consecutive low, but stable, AMH values.

As in any observational study, our findings are susceptible to confounding. The longitudinal study was conducted at two primary

schools in Copenhagen, thus we cannot exclude possible sociodemographic selection bias. All pupils were invited to participate, and we consider the participants in this study as representative of age-matched healthy Danish girls. In the final data-analysis, we have only included Caucasian girls, as racial differences in AMH levels have been suggested (Seifer *et al.*, 2009). None of the included girls had a history of gynaecological diseases or surgery, and they had not received gonadotoxic treatment or radiotherapy.

In conclusion, circulating AMH exhibits only minor fluctuations at the time of pubertal onset, and a random AMH measurement seems representative for a given girl during childhood and adolescence. The negative AMH–FSH correlation in prepubertal girls supports the notion that AMH is a quantitative marker of ovarian follicles even in young girls.

Authors' roles

L.A., K.S., K.M.M., A.M.A. and A.J. participated in the study design. C.P.H., L.A., K.S. and A.M. took part in data collection. C.P.H., L.A., K.S., A.M. and A.J. contributed to database architecture. C.P.H., A.M.A., J.H.P. and A.J. contributed to data analysis. All authors contributed to data interpretation and preparation of the manuscript.

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Conflict of interest

None declared.

References

Aksglaede L, Sorensen K, Petersen JH, Skakkebaek NE, Juul A. Recent decline in age at breast development: the Copenhagen Puberty Study. *Pediatrics* 2009;**123**:e932–e939.

Andersen CY, Schmidt KT, Kristensen SG, Rosendahl M, Byskov AG, Ernst E. Concentrations of AMH and inhibin-B in relation to follicular diameter in normal human small antral follicles. *Hum Reprod* 2010; **25**:1282–1287.

Baker TG. A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Lond B Biol Sci* 1963;**158**:417–433.

Broer SL, Eijkemans MJ, Scheffer GJ, van Rooij IA, de Vet A, Themmen AP, Laven JS, de Jong FH, Te Velde ER, Fauser BC *et al.* Anti-Müllerian hormone predicts menopause: a long-term follow-up study in normoovulatory women. *J Clin Endocrinol Metab* 2011;**96**:2532–2539.

Codner E, Iniguez G, Hernandez IM, Lopez P, Rhumie HK, Villarroel C, Rey RA. Elevated anti-Müllerian hormone (AMH) and inhibin B levels in prepubertal girls with type 1 diabetes mellitus. *Clin Endocrinol (Oxf)* 2011;**74**:73–78.

Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986;**67**:604–606.

de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Anti-Müllerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril* 2002;**77**:357–362.

Hagen CP, Aksglaede L, Sorensen K, Main KM, Boas M, Cleemann L, Holm K, Gravholt CH, Andersson AM, Pedersen AT *et al.* Serum levels of anti-Müllerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab* 2010a;**95**:5003–5010.

Hagen CP, Main KM, Kjaergaard S, Juul A. FSH, LH, inhibin B and estradiol levels in Turner syndrome depend on age and karyotype: longitudinal study of 70 Turner girls with or without spontaneous puberty. *Hum Reprod* 2010b;**25**:3134–3141.

Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertil Steril* 2011;**95**:170–175.

Hart R, Doherty DA, Norman RJ, Franks S, Dickinson JE, Hickey M, Sloboda DM. Serum anti-Müllerian hormone (AMH) levels are elevated in adolescent girls with polycystic ovaries and the polycystic ovarian syndrome (PCOS). *Fertil Steril* 2010;**94**:1118–1121.

Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER, Broekmans FJ. Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. *J Clin Endocrinol Metab* 2006;**91**:4057–4063.

Holm K, Laursen EM, Brocks V, Muller J. Pubertal maturation of the internal genitalia: an ultrasound evaluation of 166 healthy girls. *Ultrasound Obstet Gynecol* 1995;**6**:175–181.

La Marca A, Malmusi S, Giulini S, Tamaro LF, Orvieto R, Levratti P, Volpe A. Anti-Müllerian hormone plasma levels in spontaneous menstrual cycle and during treatment with FSH to induce ovulation. *Hum Reprod* 2004;**19**:2738–2741.

Pohl CR, deRidder CM, Plant TM. Gonadal and nongonadal mechanisms contribute to the prepubertal hiatus in gonadotropin secretion in the female rhesus monkey (*Macaca mulatta*). *J Clin Endocrinol Metab* 1995; **80**:2094–2101.

Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. *J Clin Endocrinol Metab* 1987;**65**:1231–1237.

Sehested A, Juul A, Andersson AM, Petersen JH, Jensen TK, Muller J, Skakkebaek NE. Serum inhibin A and inhibin B in healthy prepubertal, pubertal, and adolescent girls and adult women: relation to age, stage of puberty, menstrual cycle, follicle-stimulating hormone, luteinizing hormone, and estradiol levels. *J Clin Endocrinol Metab* 2000; **85**:1634–1640.

Seifer DB, Golub ET, Lambert-Messerlian G, Benning L, Anastos K, Watts DH, Cohen MH, Karim R, Young MA, Minkoff H *et al.* Variations in serum Müllerian inhibiting substance between white, black, and Hispanic women. *Fertil Steril* 2009;**92**:1674–1678.

Singh RP, Carr DH. The anatomy and histology of XO human embryos and fetuses. *Anat Rec* 1966;**155**:369–383.

Sorensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. *J Clin Endocrinol Metab* 2010;**95**:263–270.

Sowers MR, Eyvazzadeh AD, McConnell D, Yosef M, Jannausch ML, Zhang D, Harlow S, Randolph JF Jr. Anti-Müllerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. *J Clin Endocrinol Metab* 2008;**93**:3478–3483.

Streuli I, Fraisse T, Pillet C, Ibecheole V, Bischof P, de Ziegler D. Serum antimullerian hormone levels remain stable throughout the menstrual cycle and after oral or vaginal administration of synthetic sex steroids. *Fertil Steril* 2008;**90**:395–400.

Tanner JM. *Growth at Adolescence*. Oxford: Blackwell & Mott Ltd, 1962.

Tehrani FR, Shakeri N, Solaymani-Dodaran M, Azizi F. Predicting age at menopause from serum antimullerian hormone concentration. *Menopause* 2011;**18**:766–770.

van Disseldorp J, Faddy MJ, Themmen AP, de Jong FH, Peeters PH, van der Schouw YT, Broekmans FJ. Relationship of serum antimullerian hormone concentration to age at menopause. *J Clin Endocrinol Metab* 2008;**93**:2129–2134.

van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, Fauser BJ, Themmen AP, Te Velde ER. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. *Fertil Steril* 2005;**83**:979–987.