Anti-Müllerian hormone in short girls born small for gestational age and the effect of growth hormone treatment

A.J. Lem¹,²*, V.H. Boonstra², J.S. Renes², P.E. Breukhoven², F.H. de Jong³, J.S.E. Laven⁴, and A.C.S. Hokken-Koelega¹,²

¹Dutch Growth Research Foundation, P.O. Box 23068, 3001 KB Rotterdam, The Netherlands ²Division of Endocrinology, Department of Paediatrics, Sophia Children’s Hospital, Erasmus MC, 3000 CA Rotterdam, The Netherlands ³Division of Endocrinology, Department of Internal Medicine, Erasmus MC, 3000 CA Rotterdam, The Netherlands ⁴Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Erasmus MC, 3000 CA Rotterdam, The Netherlands

*Correspondence address. Tel: +31-10-22-515-33; Fax: +31-10-22-501-33; E-mail: a.lem@kindengroei.nl

Submitted on October 8, 2010; resubmitted on November 18, 2010; accepted on December 14, 2010

BACKGROUND: Fetal growth restriction is thought to negatively influence reproductive function in later life. Serum anti-Müllerian hormone (AMH) is a marker of the primordial follicle pool. The objectives of this study were to evaluate the effect of being born small for gestational age (SGA) on serum AMH levels and to investigate the effect of growth hormone (GH) treatment on serum AMH levels in short SGA girls.

METHODS: Serum AMH levels were investigated in 246 prepubertal girls aged 3–10 years: 119 untreated short SGA and 127 healthy controls. Associations between AMH levels and clinical characteristics were analysed using multiple regression analyses. In addition, we investigated the effect of GH treatment on serum AMH levels in short SGA girls.

RESULTS: Serum AMH levels were similar in short SGA and healthy control girls (P = 0.95). In short SGA girls, AMH levels were not significantly influenced by birth weight standard deviation score (SDS), birth length SDS and gestational age, even after adjustment for age, height SDS and body mass index (BMI) SDS at sampling, socio-economic status and maternal smoking during gestation. Serum AMH levels did not change during 4 years of GH treatment in short SGA girls (P = 0.43).

CONCLUSIONS: Serum AMH levels in prepubertal short SGA girls are similar to healthy controls, indicating that the follicle pool is not compromised due to SGA birth. GH treatment has no effect on AMH levels in short SGA girls.

Key words: anti-Müllerian hormone / small for gestational age / growth hormone / ovarian reserve / follicle pool

Introduction

Fetal life is a critical phase in the development of important organ systems, including the gonads. Already at 20 weeks of gestation, the maximum number of primordial follicles in the ovaries is reached (Block, 1953; Forabosco et al., 1991). A suboptimal intrauterine environment may have a detrimental effect on the development and preservation of primordial follicles, and may therefore impair reproductive health in later life. Granulosa cells of primary and pre-antral follicles, the stages following primordial follicles, secrete anti-Müllerian hormone (AMH) that is involved in the regulation of folliculogenesis (Weenen et al., 2004). Since serum AMH is produced exclusively by the ovaries, independently of the gonadotropic status and menstrual cycle, AMH is an excellent marker of the ovarian follicle pool (van Rooij et al., 2002; Fanchin et al., 2003; Visser et al., 2006; Kwee et al., 2008; La Marca et al., 2009; Hagen et al., 2010).

Research has been conducted to investigate the reproductive function in children with restricted fetal growth, born small for gestational age (SGA), but the results were controversial. From autopsy examination of female fetuses, the developing ovary was found to increase in size with gestational age, but did not differ between growth restricted and normal fetuses (de Bruin et al., 2001). Although some retrospective studies found higher FSH levels and reduced uterine and ovarian size in SGA girls (Ibanez et al., 2003), another group of researchers could not confirm these findings (Hernandez et al., 2006). Recently, increased levels of AMH were found in both low- and high-birth weight female infants in the first 3 months of life, compared with normal birth weight infants (Sir-Petermann et al., 2010).
Nowadays short SGA children can be treated with growth hormone (GH). Several studies showed an important role of GH, insulin-like growth factors (IGFs) and IGF binding proteins (IGFBPs) in ovarian follicular development [review (Silva et al., 2009)]. However, little is known about serum AMH levels in children treated with GH.

We hypothesised that fetal growth restriction does not affect the ovarian follicle pool and therefore SGA birth would not alter serum AMH levels in girls. To test this hypothesis, we compared serum AMH levels in a large group of prepubertal short SGA girls, with those of healthy control girls. In addition, we investigated the effect of GH treatment on the serum AMH levels in short SGA girls.

**Patients and Methods**

**SGA subjects**

The SGA group consisted of 119 prepubertal girls with short stature before start of GH treatment (aged 3–10 years). These girls were originally enrolled in Dutch multicenter GH trials (Sas et al., 1999; Arends et al., 2003; van Dijk et al., 2006; van der Kaay et al., 2009). Girls were included in the present study if they met the following criteria: (i) birth length and/or birth weight standard deviation score (SDS) for gestational age below −2.0 (Usher and McLean, 1969); (ii) height SDS for calendar age (CA) below −2.0 (Fredriks et al., 2000); (iii) height velocity SDS for CA below zero to exclude children with spontaneous catch-up growth; (iv) prepubertal stage.

Of all short SGA girls, 7.6% had partial growth hormone deficiency (GHD), defined as a maximum GH peak after stimulation test (arginine or clonidine) between 10 and 20 mU/l, and none of them had severe GHD with a maximum GH peak <10 mU/l. Girls were excluded if there was a complicated neonatal period, or signs of severe asphyxia (defined as Apgar score <3 or less after 5 min), endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness or chondrodysplasia), Turner syndrome or other syndromes (except for Silver–Russell syndrome), as well as children who were using or had used medication that could interfere with growth.

**Controls**

The control group consisted of 127 healthy girls, randomly recruited from the Erasmus MC in Rotterdam, The Netherlands. The girls were aged 3–10 years and were referred because of a minor surgical procedure. None of the girls was born preterm (gestational age <37 weeks), born SGA (birth weight <2500 g) or had a short stature (height SDS less than −1.6). Girls were excluded if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive of a yet unknown syndrome.

The studies were performed in accordance with the Helsinki declaration recommendation for conduct of clinical research and approved by the Medical Ethics Committees of the participating centres. Written informed consent was obtained from the parents or guardians of each child.

**Methods**

We analysed serum AMH levels in 119 prepubertal, short SGA girls before start of GH treatment and 127 control girls. In addition, we investigated the effect of GH on serum AMH levels in a subgroup of short SGA children. The subgroup consisted of 44 short SGA girls who were treated with GH in a dose of either 1 mg/m²/day (≏0.033 mg/kg) or 2 mg/m²/day (≏0.066 mg/kg). We compared serum AMH levels before and after a median (interquartile range, IQR) duration of GH treatment of 4.03 (2.02; 5.94) years.

Standing height, weight and Tanner stage were determined in the short SGA girls. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Height, target height (TH) and BMI were expressed in SDS, adjusting for age and gender according to Dutch reference data (Fredriks et al., 2000). Prepubertal stage was defined as Tanner breast Stage 1 (Tanner and Whitehouse, 1976). TH was calculated as $TH=([\text{maternal height}+\text{paternal height}−13]/2+4.5)$, including the secular trend of the last decades in the Dutch population. Information regarding socio-economic status (SES) and maternal smoking during gestation was obtained using questionnaires that were answered by the parents of the short SGA girls. Education level of the parents was used as socio-economic indicator to determine SES (categorized as lowest, low, medium, high; range 1–4; Verweij, 2008).

**Assays**

All samples were kept frozen until assayed (−80°C). Serum AMH levels were determined in the same laboratory by using an in-house double antibody ELISA (Kevenaar et al., 2006) or an ultra sensitive ELISA (Immunotech-Coulter, Marseilles, France) as described elsewhere (Long et al., 2000). The values of the Immunotech-Coulter assay were adjusted ($2.147$) for comparison with the in-house ELISA. The limit of detection was 0.05 μg/l. The intra- and inter-assay variation coefficients were <5 and 10% in the in-house ELISA and <5 and 8% in the Immunotech-Coulter assay. GH, IGF-I and IGFBP-3 were measured using specific radio immunoassays, as previously described (Hokken-Koelega et al., 1990). Serum levels of total IGF-I and IGFBP-3 were expressed in SDS, adjusting for age and gender, using reference values for healthy children of normal stature determined in the same laboratory (Rikken et al., 1998).

**Statistical analyses**

All data were expressed as median (IQR). SD-scores for height, TH and BMI were calculated using Growth Analyser (version 3.5; Growth Analyser b.v., Rotterdam, The Netherlands).

Due to a skewed distribution, serum AMH levels were log transformed for analyses. Power calculation was performed according to the serum AMH levels of the control group. To determine a change in mean AMH level of 1 SD, the number needed to investigate is 22 ($\alpha=0.05$ and $\beta=0.9$). Comparisons between the short SGA and the control group were conducted using the independent samples $t$-test. We used the one-sample $t$-test and $\chi^2$ test to compare variables in the SGA subgroup to the mean of the variables in the total SGA group. The paired-samples $t$-test was used to determine differences in two repeated measurements within the short SGA subgroup. The associations between serum AMH levels and clinical characteristics were analysed using multiple regression analyses. SPSS (version 16.0; SPSS Inc., Chicago, IL, USA) statistical software was used for data analysis. Results were regarded statistically significant if $P<0.05$.

**Results**

**Clinical characteristics**

The clinical characteristics of the total study group are presented in Table I. The short SGA group of 119 prepubertal girls, had a median (IQR) age of 6.24 (4.70; 7.36) years, which was similar to that of healthy controls ($P=0.72$). The SD-scores of birth weight, birth length, height at sampling, TH, BMI, IGF-I and IGFBP-3 were significantly lower than zero, the expected mean value of the control girls...
The median (IQR) serum AMH levels were similar in the short SGA group, with AMH levels before and during GH treatment, were similar to the total SGA group, except for a significant younger age at sampling (P < 0.01). The baseline characteristics of the subgroup of 44 short SGA girls, with AMH levels before and during GH treatment, were similar to the total SGA group, except for a significant younger age at sampling (P < 0.01).

### Serum AMH levels

The median (IQR) serum AMH levels were similar in the short SGA and the healthy control girls, being 5.22 (3.54; 8.19) and 4.79 (3.12; 7.82) μg/l, respectively (Table 1, P = 0.95). Figure 1 shows AMH levels against age for both groups. The majority of the short SGA girls (109/119 = 90%) had an AMH level within the normal range (between −2 and +2 SDs of controls). Serum AMH levels of short SGA and control girls were similarly distributed around the normal mean. Eight short SGA girls had an AMH level below the control −2 SDs, equivalent to 6.7% of the total SGA group, whereas 2.4% (3/127) of the control girls had, by definition, an AMH level below the −2 SDs. The percentages of girls with an AMH level below −2 SDs did not significantly differ between the short SGA girls and the controls (P = 0.13). Since serum AMH levels did not differ between partial GH deficient and other short SGA girls (P = 0.85), we analysed these two groups together.

### Growth hormone treatment

The effect of GH treatment on serum AMH levels was investigated in a subgroup of 44 short SGA girls (Table 1), who received GH for a median (IQR) duration of 4.03 (2.02; 5.94) years. Height SDS increased significantly with a median (IQR) gain in height SDS of 1.56 (1.23; 1.88; P < 0.01). Since there was no significant difference in serum AMH levels between children who received 1 or 2 mg GH/m²/day, data of both dosage-groups were analysed together. Similar serum AMH levels before and after 4 years of GH treatment were found (P = 0.43). The AMH levels were also similar to those of the control girls (P = 0.40), also after correction for age and pubertal stage at time of sampling.

### Discussion

Our study shows that serum AMH levels in prepubertal short SGA children are similar to healthy control girls, indicating that the follicle pool is not compromised due to SGA birth. We found no adverse effect of birth size on serum AMH levels, even after adjustment for

![Table 1 Clinical characteristics and serum AMH levels of the total group (short SGA and control girls) and of a short SGA subgroup.](image)
AMH in short SGA girls and the effect of GH

possible confounders as SES and maternal smoking. Subgroup analyses revealed no effect of 4 years of GH treatment on the serum AMH levels in SGA girls.

The possible effect of birth size on the ovarian follicle pool was investigated by determination of serum AMH levels in a large group of untreated short SGA girls compared with healthy control girls. Serum AMH levels were similar in both groups. Although we excluded girls born SGA from the control group, we had no exact data on birth size of this group. Therefore, we analysed possible correlations between serum AMH levels and birth size in short SGA girls. We found no correlation between serum AMH levels and birth weight SDS, birth length SDS or gestational age, even after correction for age, height SDS, BMI SDS, IGF-I SDS, IGFBP-3 SDS, SES and maternal smoking. This demonstrates that the size of the ovarian follicle pool of prepubertal short SGA girls is not reduced because of their SGA or preterm birth. Previously, reduced prenatal growth has been associated with FSH hypersecretion and reduced size of internal genitalia (Ibanez et al., 2003). However, these observations were obtained from a small and selected group. More recent studies in adolescent girls showed that fetal growth trajectories and birth size were not related to ovarian reserve (Hart et al., 2009; Kerkhof et al., 2010), in line with our results. Our study has additional value, since we analysed a much larger group of short SGA girls, and we also investigated the effect of growth hormone treatment.

Although serum AMH levels were similar in short SGA and control girls, short SGA girls might have more often an AMH level less than −2 SDS, suggesting premature ovarian failure (6.7% in the short SGA group versus 2.4% in the control group, \( P = 0.13 \)).

Syndromes with known gonadal dysfunction as Turner (Borgstrom et al., 2009) and Bloom (Chisholm et al., 2001; Kusano et al., 2001) were excluded, however, we cannot rule out that a yet unknown genetic polymorphism or mutation could be present. Nonetheless, the characteristics of the short SGA girls with an AMH below −2 SDS did not appear different from the rest of the short SGA group. Our results demonstrate that in short SGA girls, small birth size does not influence the follicle pool in the majority of the cases, although short SGA girls might be at a slightly higher risk for low AMH. Underlying problems that might result in both a short stature and a reduced ovarian reserve need further research in even larger cohorts.

In contrast to the present study, others showed increased levels of serum AMH in low birth weight infant girls. That study, however, comprised infant girls who, on average, already showed evident catch-up in weight at the age of 2–3 months (Sir-Petermann et al., 2010). We can speculate that SGA born children who show catch-up growth are more likely to have higher levels of AMH than controls, in contrast to short SGA girls. This is in line with a recent study showing higher AMH levels in young women with normal stature born SGA than in controls (Kerkhof et al., 2010).

Serum AMH reflects the ovarian follicle pool, since production of this hormone is exclusively found in granulosa cells of the pre-antral and antral follicles of the ovary (Weenen et al., 2004). Since the size and morphology of the ovaries are relatively stable during childhood (Ziereisen et al., 2005), we investigated prepubertal children in the range of 3–10 years. AMH is a good marker of the ovarian follicle pool (van Rooij et al., 2002; Kwee et al., 2008), also demonstrated in young girls (Hagen et al., 2010). The relationship with spontaneous fertility is less well-established (Levi et al., 2001) and is most often studied in adult patients with infertility (Gleicher et al.). Although the range of serum AMH levels is broad and skewed, as shown in our healthy controls, several studies demonstrated that AMH offers the clinical estimate of the ovarian reserve (de Vet et al., 2002; Barad et al., 2009; Nardo et al., 2009). Nowadays the prognostic value of serum AMH on an individual basis is similar to that of the antral follicle count, another sensitive and specific marker to predict ovarian reserve (Visser et al., 2006). Hence from the current study we conclude that reduced birth size does not alter serum AMH levels and thus ovarian reserve in prepubertal short girls.

Many children born SGA who remain short after birth are nowadays treated with GH. Since the GH–IGF-I system has an important role in oocyte fertilization (Giampietro et al., 2009), we investigated the effect of GH treatment on serum AMH levels. Our results show that AMH levels in SGA girls, who were treated with GH for a median duration of 4 years, were similar to untreated short SGA and control girls. These results indicate that GH treatment does not change the size of the ovarian pool of growing follicles in short SGA girls. Spontaneous catch-up growth after being born SGA has been associated with a higher risk of developing PCOS-like phenotype in sheep (Padmanabhan et al.). However, our results suggest that catch-up growth in height during GH treatment does not affect serum AMH levels and hence ovarian reserve.

In conclusion, prepubertal short SGA girls have similar serum AMH levels as healthy controls, indicating that the follicle pool is not compromised due to SGA birth. GH treatment has no effect on AMH levels in short SGA girls.

Figure 1 Serum AMH levels in untreated, short SGA and control girls. Serum AMH levels (µg/l) in 119 untreated, short SGA and 127 control girls (\( P = 0.95 \)). Lines represent SD-scores of control group: +2 SDS, 0 SDS, −2 SDS.
Authors’ roles

Acknowledgements
We thank research nurses C. Bruinings-Vroombout, J. Dunk, M. Huibregtse-Schouten, J. van Houten, J. van Nieuwkaesteele and E. Lems for their assistance in the studies. The participating physicians were: E.G.A.H. van Mil, Free University Hospital Amsterdam; J.C. Mulder, Rijnstate Hospital, Arnhem; R.J.H. Ondink and J.J.J. Waelkens, Catharina Hospital, Eindhoven; W.M. Bakker-van Waarde, University Medical Centre Groningen; D. Mul, W. Oostdijk and W.H. Stokvis, Leiden University Medical Centre; C. Noordam, Radboud University Medical Centre Nijmegen; C. Westerlaken, Canisius Wilhelmina Hospital, Nijmegen; N.J.T. Arends, E.M. Bannink, M. Van Dijk, D.C.M. van der Kaay, S.W.K. de Kort, Y.K. van Pareren, T.C.J. Sas and R.H. Willemsen, Erasmus Medical Centre Sophia, Rotterdam; H.M. Reeser and E.C.A.M. Houdijk, Juliana Children’s Hospital, The Hague; E.J. Schroor, Isala Clinics, Zwolle; J.P.C.M. van der Hulst, Zaans Medical Centre, Zaandam; E.J. Sulkers, Walcheren Hospital, Vlissingen; and M. Jansen, Wilhelmina Children’s Hospital, Utrecht, the Netherlands.

Funding
J.S.E.L. has received fees and grant support from the following companies (in alphabetic order); Ferring, Genovum, Merck-Serono, Organon, Schering Plough and Serono. A.C.S.H.-K. (principal investigator of the investigator responsible growth hormone studies) received independent research grants from Novo Nordisk Farma B.V. and Pfizer B.V., the Netherlands.

References
Gleicher N, Weghofer A, Barad DH. Discordances between follicle stimulating hormone (FSH) and anti-Mullerian hormone (AMH) in female infertility. Reprod Biol Endocrinol 2010; 8:64.


