

# A validated model of serum anti-Müllerian hormone from conception to menopause reflects ovarian activity throughout life.

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## Abstract

Anti-Müllerian hormone (AMH) is a product of growing ovarian follicles. Its concentration in blood may also reflect the non-growing follicle (NGF) pool (the ovarian reserve) and the rate of activation of follicle growth, and be of value in determining likely age at menopause. We have established AMH concentrations in women from age -0.3 to 59 years, using published data from normal women (n=1099), and generated a model. This was then validated using a second, independent database of similar size. The model demonstrates a rise in serum AMH to a peak at age 19.6 years, followed by a decline to the menopause, and allows the generation of normative data at all ages. During both childhood and adolescence and in adulthood, there were very close positive relationships between rate of loss of NGF pool, ie rate of initiation of follicle growth, whereas AMH showed a negative relationship to total NGF during childhood and adolescence and a positive relationship thereafter. This model indicates that AMH concentrations reflect NGF numbers and follicle growth initiation from birth to the menopause, and that there is a transition period in early adulthood when AMH starts to reflect the progressive loss of the ovarian reserve.

## Author Summary

## Introduction

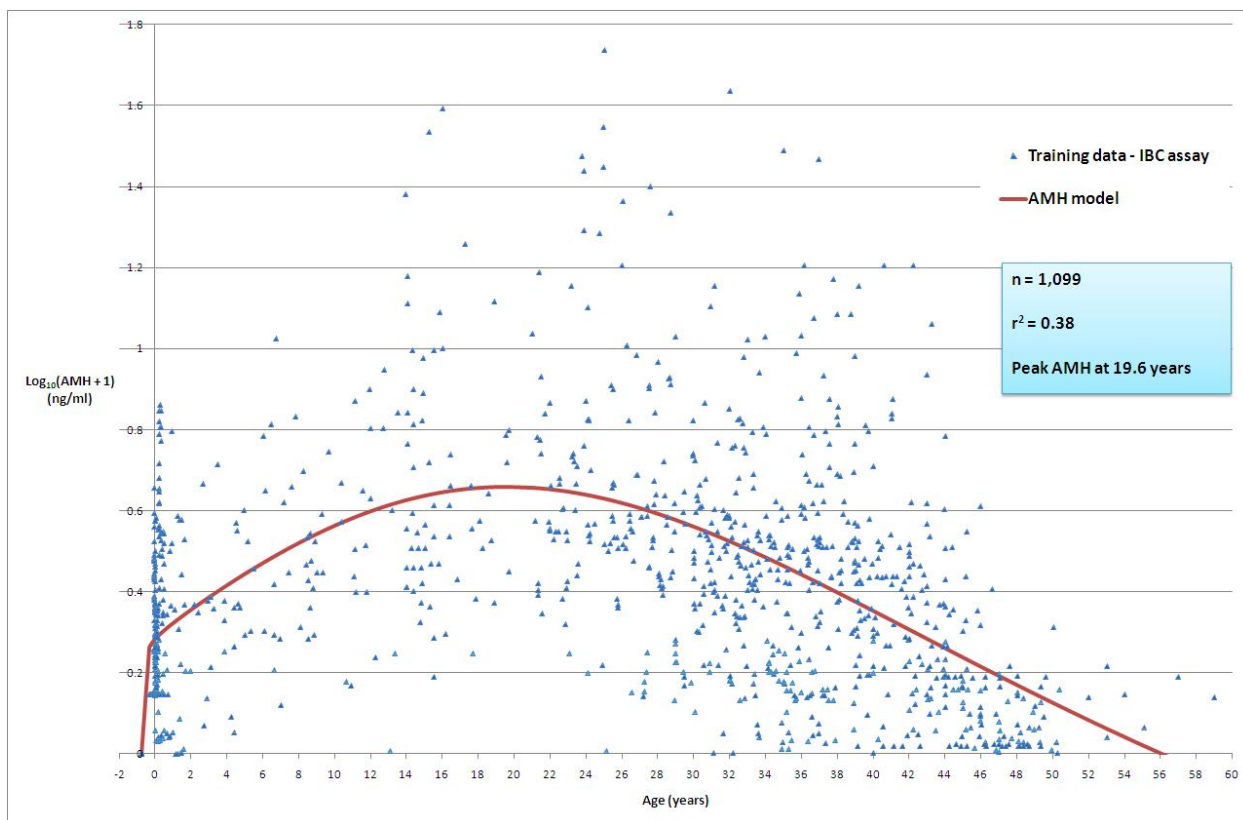
The human ovary establishes several hundred thousand non-growing follicles (NGFs) during the second half of intrauterine life, which is followed by a decline to the menopause when approximately one thousand remain at an average age of fifty to fifty-one years [1–4]. With the potential for only approximately four hundred and fifty ovulatory monthly cycles in the normal human reproductive lifespan, this progressive decline in NGF numbers is attributed to loss of growing follicles by atresia [5]. The rate of decline thus reflects initiation of follicle growth as atresia of non-growing follicles is believed to be rare physiologically. Several mathematical models have been proposed relating the decline in follicle number (based on histological analysis) to age [3, 4, 6, 7] but an accurate and non-invasive method to assess follicle number, i.e. ovarian reserve, and predict age at menopause for an individual woman remains elusive [8].

Both ultrasound examination and endocrine markers have been proposed as indirect measurements of ovarian follicle number. These include measurement of antral follicle count (AFC) and ovarian volume, and follicle stimulating hormone (FSH), inhibin B and anti-Müllerian hormone (AMH) concentrations [8]. All correlate, to different extents, with age [9] and reproductive outcome in assisted conception [10], but all are imprecise. AFC demonstrates inter-cycle variability and modest inter-observer variability [11].

Measurement of ovarian volume is imprecise, especially at low volumes [12]. Measurement of AMH may show more promise because of low intra-cycle variability and high reproducibility [13]. Furthermore AMH is the earliest endocrine marker to decrease with age and is undetectable several years in advance of the menopause [14, 15], while FSH shows little increase until the peri-menopausal period.

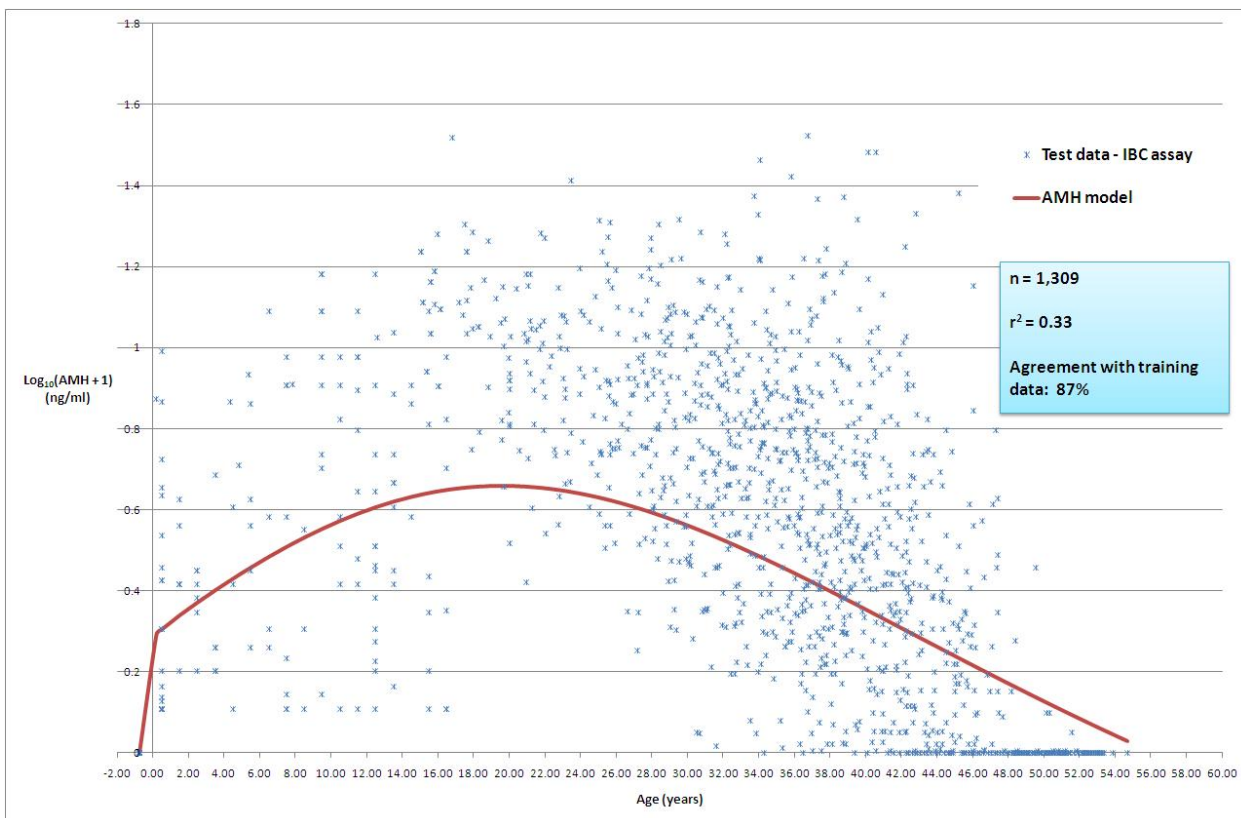
The pattern of expression of AMH is sexually dimorphic. In the male, AMH is produced by Sertoli cells from 8 weeks gestation, causing regression of the Mullerian ducts and allowing male development [16]. In the female AMH expression has been detected from 36 weeks gestation [17] but there is very little female AMH data in childhood. Several studies have shown a strong correlation between AMH and age: a prospective study showed not only that AMH declined significantly with age (a mean decrease of 38% over 3 years) [14], but that it was the only marker to do so. A similar study reported an average decrease in AMH of 58% over 4 years [18].

Many studies involving serum AMH concentrations have been based on data from subjects either having a chronic disease or problems with fertility. The aim of this study is to produce, from a systematic literature review and our own data, a validated model of serum AMH in healthy females from conception to the menopause. Having derived the validated model we can then compare correlations with NGF populations and the calculated rate of follicular recruitment.



**Figure 1. Training Data – IBC Assay** The red line is peak model that best fits the 1,099 IBC assay datapoints shown as triangles. The coefficient of determination,  $r^2$ , is 0.38, indicating that 38% of variation in serum AMH levels is due to age alone. Peak serum AMH is at 19.6 years.

## Results



**Figure 2. Test Data – DSL Assay** The red line is the peak model from Figure 1, fitted to the 1,309 DSL datapoints shown as crosses. The coefficient of determination,  $r^2$ , is 0.33 which is 87% of the  $r^2$  for the training data.

### Training (IBC assay) data

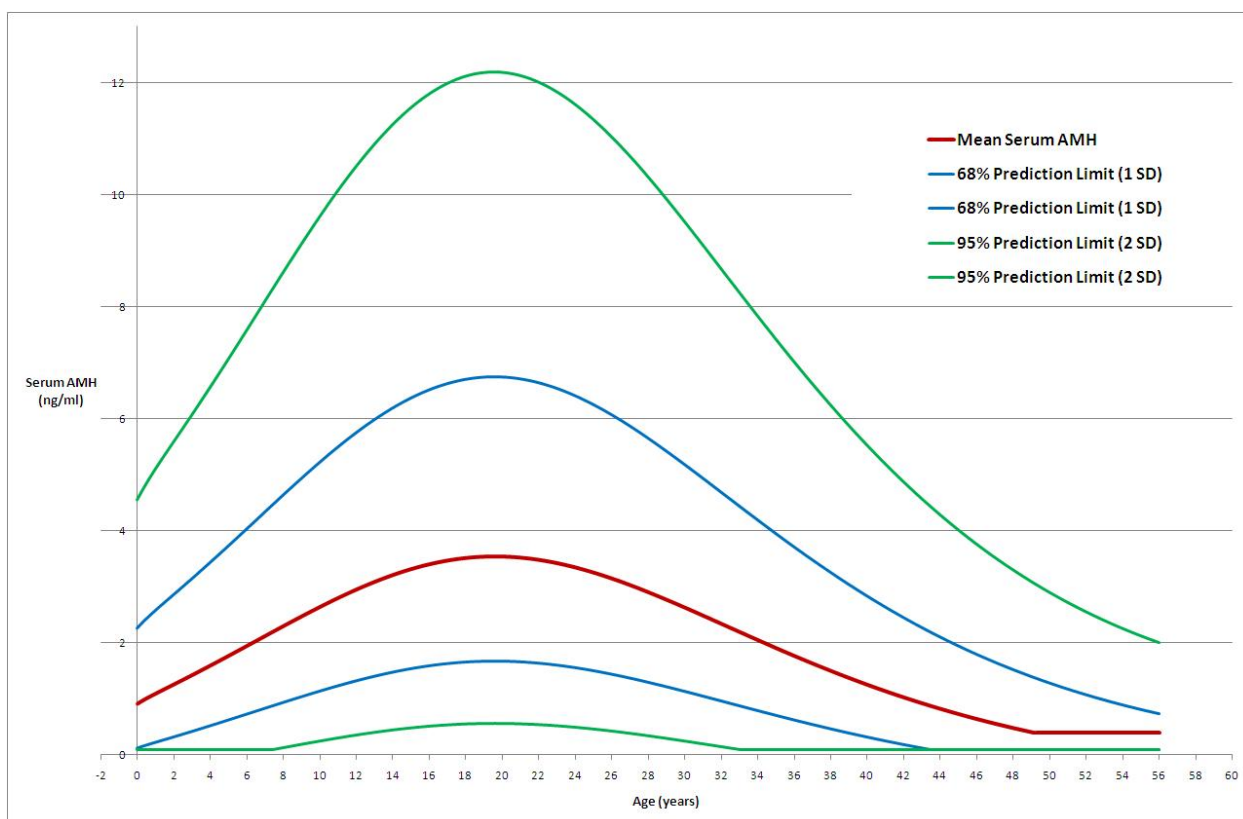
Data obtained from published studies using the IBC assay were used to derive the model. This included 1099 data points across the age range from -0.3 (cord blood from preterm infants) to 59.0 years. The maximum  $r^2$  returned for any of the 268 TableCurve 2D models was 0.41. Overall, modelling showed increasing AMH concentrations until adolescence/early adulthood with a progressive decline thereafter. The best peak model from conception to 59 years was of the form

$$\log_{10}(AMH + 1) = \frac{\alpha + \gamma * \text{age} + \epsilon * \text{age}^2}{1 + \beta * \text{age} + \delta * \text{age}^2}$$

with  $\alpha = 0.283$ ,  $\beta = 1.246$ ,  $\gamma = 0.402$ ,  $\delta = -0.019$  and  $\epsilon = 0.033$ . This had an  $r^2$  of 0.38, which is 93% of the maximum obtainable for any model, and a peak at 19.6 years. Several other models returned similar  $r^2$  values and had similar peaks at 18–20 years. Figure 1 shows the training data and the best peak model.

## Validation (DSL assay) data

A second independent dataset was used to validate the training model. Data collection generated a similar number of data points in the literature deriving from the use of a second immunoassay, the DSL assay (n=1309, age range 0.2 to 54.7 years). The  $r^2$  for the test (DSL assay) analysis derived from residuals from the best peak model for the training data was 0.33 (Figure 2), which is in good agreement – 87% – with the 0.38 coefficient of determination for the training data. Since the two datasets are independent (and given that the conversion factor used is known to be imprecise with 85% accuracy across the physiological range of AMH concentrations [19]) we consider that the best training model generalises well to unseen data, and hence report this as a validated model for serum AMH levels in the normal female population (Figure 3).



**Figure 3. The normal range for serum AMH in girls and women** The red line is the log-unadjusted validated AMH model. The blue and green lines are the 68% and 95% prediction limits for the model (plus and minus one and two standard deviations respectively).

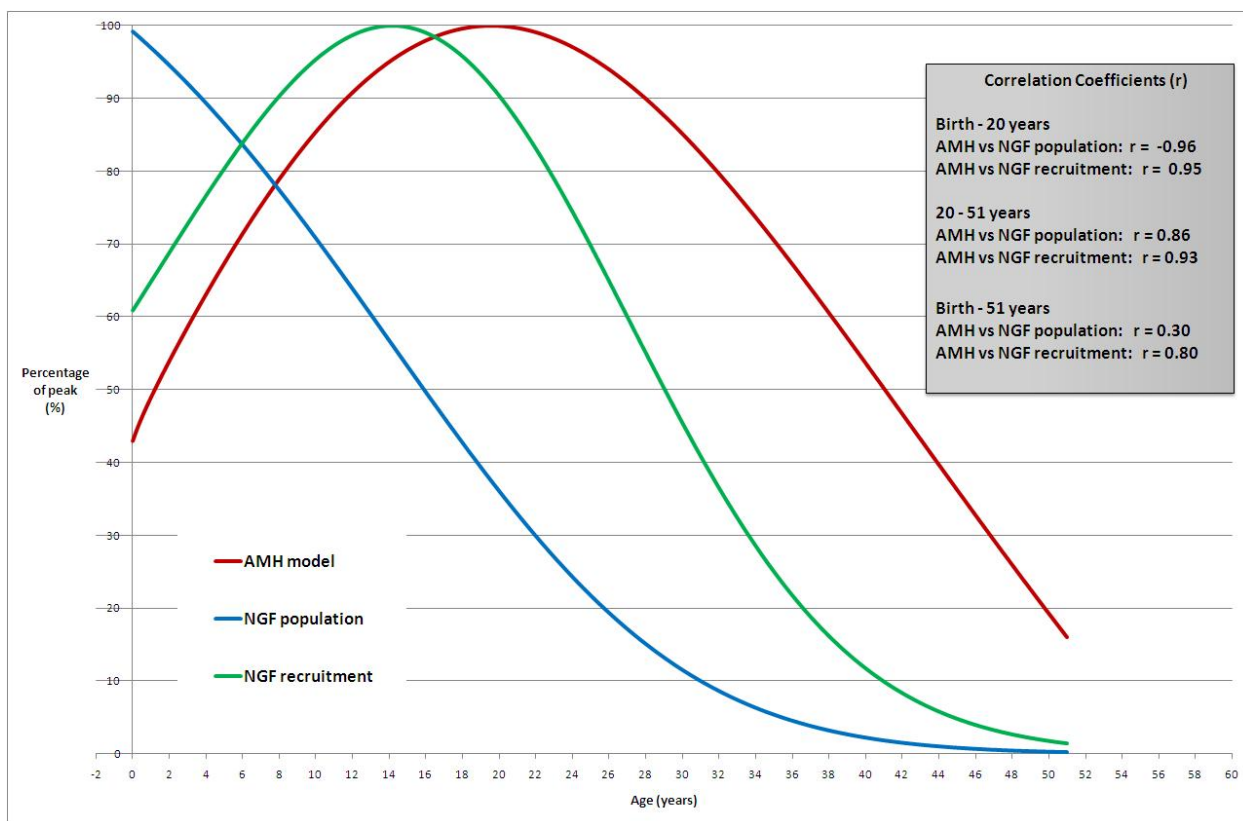
## Comparison with NGF population models

All ovarian follicles are formed before birth, and their number is already declining by that time. We therefore performed two initial analyses to compare NGF and AMH data, one during childhood and adolescence when NGF is falling and AMH is rising, and a second from the time of peak AMH concentration until the menopause. We found that from birth to 20 years NGF population is negatively correlated

with AMH ( $r = -0.96$ ), since NGF populations are falling while AMH levels are rising. However over the same age range AMH is closely and positively correlated with rate of NGF recruitment ( $r = 0.95$ ). AMH increases in parallel with NGF recruitment, but with a delay of about 5 years (Figure 4).

Different but comparably close relationships were found between AMH concentrations and NGF populations from early adulthood until the menopause. From 20 to 51 years both NGF population and NGF recruitment correlate well and positively with AMH ( $r = 0.86$  and  $r = 0.93$  respectively).

We finally analysed AMH and NGF populations from birth to the menopause (51 years). Over this timecourse the NGF population correlated only poorly with AMH ( $r = 0.30$ ), but there remained a close positive correlation between NGF recruitment and AMH ( $r = 0.80$ ).



**Figure 4. Comparison of serum AMH levels with NGF population and with NGF recruitment** The red line is the log-unadjusted validated AMH model, peaking at 19.6 years. The blue line denotes the decline in NGF population [4], with peak population at 18-22 weeks gestation. The green line denotes the numbers of NGFs recruited towards maturation [4], with peak numbers lost at age 14.2 years on average. Each quantity has been normalised so that the peak occurs at 100%. Correlation coefficients ( $r$ ) are given for AMH levels against the other two curves for birth to 20 years, 20 to 51 years, and birth to 51 years.

## Discussion

We report the first model of serum AMH in the normal, fertile human population from conception to menopausal ages (Figure 1). This model was obtained from a set of data from over 1000 subjects with ages ranging from -0.3 years to 59 years. The model was validated using an independent set of data with a similar number of subjects, average age and range of ages, with similar correlations of determination obtained for the model applied to the two datasets (Figure 2). This close agreement in  $r^2$  values suggests that the model will accurately describe unseen data, and therefore accurately predicts levels of serum AMH in females (Figure 3).

Further analysis shows that serum AMH level correlates well with ovarian reserve but that this relationship is dramatically different at different stages of life (Figure 4). During childhood and adolescence AMH concentrations are rising while NGF number is declining. However during this period we have previously demonstrated that follicle recruitment is in fact also increasing (KW 2010), thus is positively and closely related to AMH concentration across this age range. A very different relationship holds during adult life, with AMH and NGF number declining together, with NGF recruitment and loss also declining. This latter finding is in accord with numerous cross-sectional studies showing that AMH concentrations fall with age, and are predictive of oocyte (ie growing follicle) number following superovulation for assisted conception [11, 20, 21]. That AMH concentrations are related to NGF number has recently been demonstrated in a study of histological specimens from adult women [22], supporting rodent data [23].

This model demonstrates that serum AMH levels peak at age 18-20. This is consistent with a study published in 2002 that shows AMH decreasing after age 20 [14], however two more recent studies have reported peak AMH at age 31-33 [24, 25]. The minimum age for subjects in these studies was 14 years and 25 years respectively. We speculate that lack of data from younger (especially neonatal) subjects has skewed the reported peaks towards a higher age. This finding has important implications for our understanding of ovarian biology. The prepubertal ovary shows follicle growth to the early antral stage whereas after puberty increased gonadotrophin secretion (particularly FSH) from the pituitary gland allows the initiation of complete follicular maturation and the onset of ovulatory cycles. AMH is produced by granulosa cells, with expression initiated at the onset of follicle growth with a sharp decline as follicles attain a diameter of approximately 10mm [26], which is the size at which selection for ovulation occurs. The mechanisms involved in the regulation of these processes are unclear, but AMH is thought to be an important inhibitor of the growth of early follicles, acting in a paracrine manner on nearby follicles [27]. The present analysis indicates that there is a transition period between puberty and age 20, during which time there is increasing growth of the full complement of ovarian follicles following shortly after the peak in NGF recruitment, reflected in the peak in AMH secretion, whereafter the relationship with the total number of follicles present, the NGF population, changes from a negative one to a positive one with both then declining to the menopause.

These data provide a novel understanding of the rise and fall of AMH from birth through puberty to menopause. This information advances our knowledge of the assessment of ovarian reserve for the individual woman and provides important insights for the further assessment of normal and disordered ovarian physiology.

## Materials and Methods

### Data acquisition

Studies involving serum AMH measurements of human females were identified by performing PubMed and Medline searches and searching individual journals (including Menopause, Fertility and Sterility, Human Reproduction and the Journal of Clinical Endocrinology and Metabolism) using the search terms AMH, Mllerian inhibiting substance, ovarian reserve and polycystic ovarian syndrome. The references

of included studies (Table One; [14, 18, 24, 25, 28–41]) were checked to identify further relevant studies to be processed. Data was selected for this analysis only for subjects who were not known to be infertile or have an identified illness (eg Cancer, SLE) being either the control groups from controlled studies or subjects from prospective studies of the healthy population. Any data from subjects with a chronic disease or undergoing infertility assessment or investigation were excluded from the study. Data from the cord blood of 53 infants [28] were included. The data were extracted from graphs using Plot Digitizer software [42] to convert datapoints on the graphs into numerical data. Repeated datapoints were isolated by requiring that the acquired dataset matched the descriptive statistics provided in the supporting paper (Table One).

We combined the resulting dataset with two sets of raw Scottish data. The first consisted of individual serum AMH measurements (n=441, median age 36.1 years, max. age 47.8, min age 21.9) undertaken between July 2006 and November 2009 in the biochemical laboratories of the Glasgow Centre for Reproductive Medicine (Table One). This cohort consists of women whose partners were known to have severe male factor infertility requiring ICSI and where no other female cause of infertility had been identified. The second dataset was supplied by Ahmed et al. [41] and consists of 128 measurements taken from subjects aged 0.5 – 16.5 years.

Serum AMH values were standardised to give AMH measurements in ng/ml using the conversion formula  $1 \text{ pmol/l} = 7.143 \text{ ng/ml}$ .

## Data analysis

The resulting data were split into two sets depending on the assay used to obtain serum AMH values. The first dataset (n=1099, median age 29.5 years, max. age 59.0, min age -0.3) came from those studies in which the serum concentrations of AMH were determined using enzyme-linked immunoassay kits IBC (Immunotech Beckman Coulter Company, France). The second dataset (n=1309, median age 35.4 years, max. age 54.7, min age 0.2) came from studies in which the enzyme-immunometric assay Active MIS/AMH ELISA kits DSL (Diagnostic Systems Laboratories Inc., TX, USA) were used. Since the two sets have similar statistical properties and are independent of each other we performed modelling using the IBC values as a training set, with validation analysis – i.e. how well the best training data model generalises to unseen data – performed taking the DSL values as test data.

For the training set we added zero values at conception in order to force models through the only known AMH concentration at any age. As variability increases with AMH concentration, we log-adjusted the data (after adding one to each value so that zero AMH on a chart represents zero serum AMH). We fitted 268 mathematical models to the resulting test data using TableCurve-2D (Systat Software Inc., San Jose, California, USA), and ranked the results by coefficient of determination,  $r^2$ . Each model defines a generic type of curve and has parameters which, when instantiated gives a specific curve of that type. For each model we calculated values for the parameters that maximise the  $r^2$  coefficient. The Levenberg-Marquardt non-linear curve-fitting algorithm was used throughout, with convergence to 5 significant figures after a maximum of 1,000 iterations. Many of the models are not biologically plausible, in the sense that they predict serum AMH rising and falling repeatedly throughout life. We report the highest  $r^2$  for any model, but choose as the most biologically plausible model for this data the highest ranked model that peaks exactly once (the first derivative changes sign once) and is convex (has negative second derivative throughout) in the age range from conception to 54 years: a peak model.

At the validation stage we converted the DSL data into IBC values using the conversion formula

$$2.02 * DSL = IBC$$

[19], which has a reported  $r^2$  of 0.85. The resulting datapoints had the same proportion (10%) of zeros at conception added as for the training data, were log-adjusted, and residuals from the best training model

were derived for each datapoint. The  $r^2$  coefficient of determination was derived using the formula

$$r^2 = 1 - \frac{SSE}{SSM}$$

where  $SSE$  is the sum of the squared residuals and  $SSM$  is the sum of the squared differences from the mean of the test data AMH values. Degree of validation of the training data model by the test data was obtained by comparing the  $r^2$  for each of the datasets.

We correlated the validated AMH model with existing models of both NGF population and number of NGFs lost through recruitment towards maturation [4]. Since the AMH model peaks at age 19.6, we correlated in each case from birth to age 20; from age 20 to age 51 years (the highest age for the NGF models [4]); and from birth to 51 years.

## Tables

**Table 1. Serum AMH data summary**

Ref.	1 <sup>st</sup> Author	Data	Assay	$n$	Average age	Range	Det. limit	Intra CV	Inter CV
[24]	Soto	Graph	IBC	58	30.3 (mean)	$\pm 8.7$ SD	0.10	5.3	8.7
[28]	Guibourdenche	Graph	IBC	192	NS	-0.3 – 1.0	0.30	5.3	8.7
[29]	Hudecova	Graph	IBC	64	46.3 (mean)	$\pm 6.4$ SD	0.70	12.3	12.3
[30]	Mulders	Graph	IBC	82	29.9	19.6 – 35.6	NS	5.0	8.0
[31]	Pastor	Graph	IBC	42	NS	18.0 – 50.0	0.10	5.3	7.8
[32]	Piltonen	Graph	IBC	44	31.6 (mean)	21.0 – 44.0	NS	5.1	6.6
[18]	van Rooij	Graph	IBC	162	NS	25.0 – 46.0	0.05	5.0	8.0
[33]	Laven	Graph	IBC	41	NS	20.0 – 36.0	0.05	5.0	8.0
[14]	de Vet	Graph	IBC	82	29.0	$\pm 4.0$ SD	0.05	5.0	8.0
[34]	Knauf	Graph	IBC	83	34.2 (mean)	$\pm 3.4$ SD	0.03	11.0	11.0
[35]	Lee	Graph	IBC	225	NS	0.0 – 51.0	0.50	9.0	15.0
[36]	La Marca	Graph	IBC	24	44.0 (mean)	$\pm 2.8$ SD	0.24	5.0	8.0
[37]	van Beek	Graph	DSL	82	29.0	20.0 – 35.0	NS	5.0	15.0
[38]	Sanders	Graph	DSL	43	24.1 (mean)	0.1 – 51.0	0.01	NS	11.4
[25]	van Disseldorp	Graph	DSL	144	37.9 (mean)	25.0 – 46.0	0.03	11.0	11.0
[39]	Tehrani	Graph	DSL	267	27.1	16.0 – 44.0	0.01	5.2	9.1
[40]	Dorgan	Graph	DSL	204	44.7 (mean)	33.3 – 54.7	0.06	8.0	8.0
[41]	Ahmed	Raw	DSL	128	8.5	0.5 – 16.5	0.50	8.0	8.0
	Nelson	Raw	DSL	441	36.1	21.9 – 47.8	0.03	3.4	8.6
	<b>Total IBC</b>			<b>1,099</b>	<b>29.5</b>	<b>-0.3 – 59.0</b>			
	<b>Total DSL</b>			<b>1,309</b>	<b>35.4</b>	<b>0.2 – 54.7</b>			
	<b>Total n</b>			<b>2,408</b>	<b>32.0</b>	<b>-0.3 – 59.0</b>			

The references relate to the bibliography section of this paper. Average ages in years are medians unless stated otherwise; SD denotes standard deviations. Detection limits are given in ng/ml; intra- and inter-observer coefficients of variation (CV) are percentages. All descriptive statistics are those reported in the referenced study; NS denotes not stated. For longitudinal studies – [14, 30, 39] – we report the average age of participants at first measurement.

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