

The physiology and clinical utility of anti-Mullerian hormone in women

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64 **Introduction**

65

66 The physiology and clinical utility of anti-Müllerian hormone (AMH) are not completely established.
67 However, because of the tremendous amount of data collected in recent years, it appeared timely for
68 this group of experts to bring together the current knowledge. These experts met in Lille, France, in May
69 2012 for an ESHRE Campus workshop. This review offers a structured proceeding of this workshop that
70 has been updated with the most recent data published in the literature since then. Its aim is to provide
71 an extensive overview of the current knowledge and position of AMH as a tool in female health and
72 fertility care. [While covering most aspects of the physiology and utility of AMH, some aspects \(e.g., use
73 in diagnosis of granulosa cell tumours\) were not covered, but are discussed in excellent reviews \(e.g., La
74 Marca et al., 2007\).](#)

75

76 **Historical perspective and state of the art**

77

78 AMH is a dimeric glycoprotein and a member of the transforming growth factor β (TGF- β) family of
79 growth and differentiation factors (Cate et al., 1986). AMH has been predominantly known for its role in
80 male sexual differentiation. From castration experiments in the fetal rabbit, Jost demonstrated that a
81 testicular factor distinct from testosterone was responsible for the regression of the Müllerian ducts
82 during male fetal sex differentiation (Jost, 1947). In later years, it was demonstrated that this factor is
83 produced by Sertoli cells in the testis (Josso et al., 1993).

84 The ovary is also able to produce AMH. In the chicken, this occurs from early embryonic development to
85 adulthood (Hutson et al., 1981) but in human, AMH production by granulosa cells was detected only at
86 the end of gestation (Rajpert-De Meyts et al., 1999). Interest into the role of AMH in the female was
87 principally evoked through studies of AMH-deficient mice. Although female mice appeared fertile in the
88 absence of AMH (Behringer et al., 1994), more detailed analysis of the ovarian follicle pool revealed that
89 AMH acted as an inhibitor of primordial follicle recruitment. Also, later studies demonstrated a potential
90 role for AMH in dominant follicle selection in the follicular phase of the menstrual cycle (Visser and
91 Themmen, 2005). The development of sensitive assays soon enabled measuring AMH in serum (Hudson
92 et al., 1990; Josso et al., 1990; Baker et al., 1990). Release of AMH from the granulosa cells of antral
93 follicles leads to measurable serum levels, and these concentrations have shown to be proportional to
94 the number of developing follicles in the ovaries. Therefore, AMH was considered to be a marker for the
95 process of ovarian ageing (Kevenaar et al., 2006).

96 To date, AMH has developed into a factor with a wide array of clinical applications, mainly based on its
97 ability to express the number of antral and pre-antral follicles present in the ovaries (Hansen et al.,
98 2011). Predicting ovarian response to hyperstimulation of the ovaries for IVF, with the possibility of
99 individualized counseling and adjustments of the stimulation regimen, is the most appealing application
100 under development so far. Assessment of damage to the ovarian follicle reserve inflicted by iatrogenic

101 sources such as pelvic irradiation, chemotherapy, uterine artery embolization or ovarian surgery using
102 AMH may open avenues of choosing strategies to prevent this damage in selected cases by applying
103 fertility preservation strategies. The emerging data on the relation between AMH level at a certain age
104 and the timing of menopause has set a scene for an individualized prediction of the reproductive
105 lifespan, and from there potential prevention of infertility based on early ovarian ageing. Finally,
106 marking the excess of antral follicles in women with Polycystic Ovarian Syndrome (PCOS), AMH may
107 soon replace the ultrasound ovarian morphology criterion in the diagnosis of this syndrome, as well as
108 become an additional tool for diagnosing premature ovarian insufficiency (POI).

109 **Physiology**

110 The roles of AMH in ovarian physiology

111

112 AMH is specifically expressed in granulosa cells of small growing follicles. In rodents, expression is
113 initiated as soon as primordial follicles are recruited to grow, and highest expression is observed in
114 preantral and small antral follicles. AMH is no longer expressed by mural granulosa cells during the FSH-
115 dependent stages of follicular growth, nor is it expressed in atretic follicles (reviewed in Durlinger et al.,
116 2002). However, expression persists in the cumulus cells of preovulatory follicles (Salmon et al., 2004). In
117 the human ovary, AMH shows a very similar expression pattern (Rey et al., 2000; Weenen et al., 2004;
118 Grondahl et al., 2011; Jeppesen et al., 2013).

119 Functional roles of AMH in ovarian folliculogenesis were revealed by analysis of the follicle pool in
120 ovaries of AMH-deficient mice at various ages. In the absence of AMH, primordial follicles are recruited
121 at a faster rate, resulting in an exhausted primordial follicle pool at a younger age (Durlinger et al.,
122 1999). The inhibitory effect of AMH on primordial to primary follicle transition was confirmed by *in vitro*
123 studies of neonatal ovaries and ovarian cortical strips of various species, including human (Durlinger et
124 al., 2002; Nilsson et al., 2007; Gigli et al., 2005; Carlsson et al., 2006). However, contradictory results
125 using human ovarian cortical tissue have also been reported (Schmidt et al., 2005). In the mouse AMH
126 inhibited the effect of several growth factors known to have a stimulatory action on primordial follicle
127 recruitment, such as KitL and bFGF (Nilsson, et al., 2007). In the absence of AMH, ovaries contain more
128 growing follicles, yet AMH-deficient mice have a normal ovulation rate. Increased oocyte degeneration
129 and follicular atresia suggests that AMH may also be a survival factor for small growing follicles (Visser et
130 al., 2007). AMH also reduces follicle sensitivity to FSH *in vivo*, and *in vitro* AMH inhibited FSH-induced
131 preantral follicle growth (Durlinger et al., 2001). Thus, there is clear evidence that AMH is involved in the
132 regulation of follicle growth initiation and the threshold for FSH sensitivity (Figure 1).

133 AMH has also been suggested to exert a physiological effect on antral follicles in the human ovary
134 before final selection. There exists a fine-tuned and delicate balance between estradiol (and inhibin)
135 output by the preovulatory follicle and gonadotrophin secretion by the pituitary to ensure that ovulation

136 is triggered exactly at the right time (Baird and Smith, 1993). Recently, it has been suggested that AMH
137 may exert a physiological role in down regulating the aromatizing capacity of granulosa cells until the
138 time of follicular selection (Figure 1). Several studies have shown that AMH expression remains high
139 until a follicle reaches a diameter of around 8mm (Weenen, et al., 2004, Andersen et al., 2010,
140 Jeppesen, et al., 2013). The intrafollicular concentrations of AMH in normal human antral follicles show
141 a gradual reduction as the diameter of the follicle increases, and a sharp decline is observed around
142 8mm (Andersen, et al., 2010). The rapid decline in AMH expression corresponds with the selection of
143 follicles for dominance, which is characterized by a transition from a low-estrogen producing state to
144 one of rapidly increasing estrogen production. Estradiol is instrumental in this decline through estradiol
145 receptor β , which interacts with the AMH promoter region (Grynberg et al., 2012) (Figure 1).

146 Several lines of evidence suggest that AMH acts as gatekeeper of follicular estrogen production:

- 147 1) Early studies on fetal ovine ovaries showed that AMH repressed aromatase biosynthesis (Vigier
148 et al., 1989). A quantitative bioassay for AMH was subsequently developed based on inhibition
149 of cAMP-induced aromatase activity in fetal rat ovaries (di Clemente et al., 1992).
- 150 2) In granulosa-lutein cells from IVF patients AMH reduces the expression of CYP19a1 at both gene
151 and protein level and FSH-induced E2 production was significantly reduced in the presence of
152 AMH (Grossman et al., 2008).
- 153 3) In human small antral follicles there is a distinct inverse association between intrafollicular
154 concentrations of AMH and estradiol concentrations and *CYP19a1* gene expression in the
155 corresponding granulosa cells (Andersen and Byskov, 2006, Andersen and Lossl, 2008, Nielsen et
156 al., 2011).
- 157 4) Using adjacent ovarian sections of preovulatory sheep follicles it was observed that the oocyte
158 cumulus complex showed an almost complete inverse expression pattern of AMH and
159 aromatase (Campbell et al., 2012). AMH continues to be expressed in cumulus cells of
160 preovulatory follicles in the human (Grondahl, et al., 2011).
- 161 5) Association analysis of genetic variants of the AMH signaling pathway showed that the AMH
162 Ile⁴⁹Ser and AMH type 2 receptor (AMHR2) -482A>G variants were related to follicular-phase
163 estradiol levels in normo-ovulatory women. Women carrying the minor allele of the AMH or the
164 AMHR2 polymorphism had higher estradiol levels compared to non-carriers, with carriers of
165 both minor alleles having the highest levels (Kevenaar et al., 2007). *In vitro*, the AMH⁴⁹Ser
166 variant yields a less active AMH protein that could result in weaker inhibition of FSH-induced
167 aromatase activity and follicle growth (Kevenaar et al., 2008).

168 Thus, AMH may act as a follicular gatekeeper and ensure that each small antral follicle produces little
169 estradiol prior to selection (i.e. up to a follicular diameter of approximately 8mm) allowing a direct
170 ovarian/pituitary dialogue regulating the development of the selected follicle that will undergo
171 ovulation (Jeppesen, et al., 2013) (Figure 1).

172 **Assessment of AMH in serum: assay development**

173 AMH is produced as a precursor protein, consisting of 70 kDa disulphide-linked monomers (Picard and
174 Josso, 1984). Proteolytic processing yields a 55 kDa N-terminal proregion and a 12.5 kDa C-terminal
175 mature region (Pepinsky et al., 1988, Nachtigal and Ingraham, 1996). The pro- and mature homodimers
176 remain non-covalently associated, resulting in a 140 kDa complex in circulation (Lee and Donahoe, 1993)
177 (Figure 2). The mature region of AMH holds the biological activity of the protein, but in contrast to other
178 TGF β family members, requires the N-terminal proregion to obtain its full activity (Wilson, 1993). It has
179 been suggested that the proregion is involved in protein stability and folding (Belville et al., 2004).

180 Measurement of serum AMH was first reported in the 1990s, with the development of three AMH
181 enzyme-linked immunosorbent assays (ELISAs) (Baker et al., 1990; Hudson, et al., 1990; Josso et al.,
182 1990). The AMH ELISAs were initially developed to measure AMH as a marker for testicular function
183 during childhood, when serum concentrations are much higher than in females. Using a monoclonal and
184 a polyclonal antibody that were both raised against recombinant human AMH (rhAMH), and which both
185 recognize epitopes in the proregion of AMH, a sensitivity of 0.5 ng/ml was reached (Hudson, et al.,
186 1990). Baker *et al* developed an assay with antibodies raised against bovine AMH and rhAMH, but this
187 assay was unable to detect AMH in female serum samples because of the relatively high detection limit
188 of 6.25 ng/ml and the presence of inhibitory effects of serum. The assay developed by Josso and
189 colleagues used a single polyclonal antibody raised against purified bovine AMH with rhAMH as the
190 standard (Josso, et al., 1990). In this assay, the minimal detectable dose of AMH was 0.02 ng. This assay
191 was subsequently modified to a sandwich ELISA using a monoclonal and polyclonal antibody raised
192 against rhAMH. These antibodies recognize epitopes in the pro- and mature region of AMH (Figure 2),
193 and increased the sensitivity of the assay to 14 pmol/L (1 ng/ml = 7.14 pmol/L) (Carre-Eusebe et al.,
194 1992). A further improvement in sensitivity to 0.7 pmol/L was reached by the use of two different
195 monoclonal antibodies (Long et al., 2000). This ultrasensitive assay, known as the IOT assay, became
196 commercially available through Beckman-Coulter (originally Immunotech-Coulter).

197 The importance of assessment of serum AMH levels in females followed the insight (based on the
198 expression pattern) that serum AMH might be a proxy for the size of the primordial follicle pool
199 (reviewed in Visser et al., 2006). This led to the development of an additional sensitive AMH ELISA.
200 Highly specific monoclonal antibodies to the proregion of AMH were generated by immunization of
201 female AMH-deficient mice with rhAMH (Al-Qahtani et al., 2005). These antibodies had different
202 epitope specificities and, with rhAMH as the standard, the detection limit improved to 0.078 ng/ml (Al-
203 Qahtani, et al., 2005). This assay was subsequently improved with another pair of highly specific
204 monoclonal antibodies, which recognize epitopes in both the proregion (F2B/7A) and mature regions
205 (F2B/12H) (Kevenaar, et al., 2006) (Figure 2). This assay is therefore expected to measure total AMH,
206 and was commercially available through Diagnostic Systems Lab (DSL), has a detection limit of 6.3 pg/ml
207 (Kevenaar, et al., 2006).

208 With the availability of two commercial assays, research on the clinical application of serum AMH
209 increased tremendously. However, because these assays use different antibody pairs, and even more
210 importantly different AMH calibrators, values of serum AMH differed significantly between the assays
211 being 3- to 4- fold lower in the DSL assay (Freour et al., 2007). In later publications, similar AMH values
212 were reported for both assays (Streuli et al., 2009, Lee et al., 2011), indicating that the assays continued
213 to evolve. This may, in part, explain the different conversion factors that have been reported in various
214 studies (Hehenkamp et al., 2006, Freour, et al., 2007). As a consequence, values obtained by one assay
215 may not be directly translated to results obtained with the other assay.

216 With the acquisition of DSL by Beckman-Coulter, the two existing assays were replaced by a new ELISA.
217 This Beckman-Coulter AMH Gen II assay continues to use the antibodies of the previous DSL assay but
218 uses native AMH in heat-inactivated bovine calf serum as a standard. The Gen II assay was calibrated to
219 the IOT AMH ELISA, yielding a sensitivity of 0.08 ng/ml (Kumar et al., 2010). Comparison of the AMH Gen
220 II assay with the previous assays showed that AMH values obtained with the AMH Gen II assay had a
221 good correlation with those of the DSL assay but higher values (22-40%) were obtained with the Gen II
222 assay (Wallace et al., 2011, Li et al., 2012). Because the AMH Gen II assay was calibrated to the IOT
223 assay, this difference could potentially be accounted for by the previously observed difference between
224 the DSL and IOT assays. However, Li et al also observed a 35% increase in sample value in the Gen II
225 assay compared to the IOT assay (Li, et al., 2012). This finding is unexpected given that the AMH Gen II
226 assay was calibrated to the IOT assay. Furthermore, there have been studies questioning the stability of
227 AMH upon storage, sample handling and sample diluting, either prior to or by sequential addition to the
228 microtitre plate, which all might affect serum AMH values (Rustamov et al., 2012). **In contrast, stable**
229 **serum AMH values were reported upon long term storage at -20°C with the previous DSL assay**
230 **(Kevenaar, et al., 2006). Also with the AMH Gen II assay fairly stable values were reported for serum**
231 **AMH but not for whole blood (Kumar, et al., 2010, Fleming and Nelson, 2012, Fleming, et al., 2013).**
232 Concerns about the robustness of the AMH Gen II assay have been fuelled by recent safety notices and
233 technical update letters from Beckman-Coulter, indicating that undiluted samples may give falsely low
234 values due to interference from complement, but also that some samples diluted prior to addition to the
235 plate may give falsely elevated values. Therefore, results published so far with the AMH Gen II assay
236 have to be taken with caution and will probably need to be revisited once the technical issues are
237 resolved. **Furthermore, it is recommended that these changes are validated in independent research**
238 **before clinical application of the assay.** Adapting clinical cut-off values from the IOT assay to the Gen II
239 assay is not recommended, because a different antibody pair is used. **Likewise, a simple conversion**
240 **factor to recalculate values from the DSL assay to AMH Gen II is also not recommended, given the issues**
241 **raised above. Therefore, although the clinical application of serum AMH, as discussed in this review, is**
242 **not in question, it is also not recommended to compare absolute values from clinical studies that use**
243 **different assays.** To maximise the clinical utility of AMH measurement it is also critical to develop an

244 international standard for AMH that is safeguarded and distributed by a competent authority such as
245 the National Institute for Biological Standards and Control. This would allow harmonization of current
246 and potential new AMH assays, thereby eliminating the need to establish assay-specific normative and
247 cut-off values.

248

249 **Variability of serum AMH in normal women**

250

251 Inter-individual variability of AMH is high, mainly due to the very high variability in the number of antral
252 follicles within groups of subjects of similar age (Gougeon, 1998; La Marca et al., 2011; Almog et al.,
253 2011). There also seems to be ethnic variation, with African-American (Seifer et al., 2009; Schuh-Huerta
254 et al., 2012) and Hispanic (Seifer, et al., 2009) women having lower serum AMH levels than those found
255 in Caucasian women which may indicate a discrepancy between ovarian follicle number and AMH
256 production. Some studies have indicated a negative relationship between BMI and AMH (Freeman et al.,
257 2007; Steiner et al., 2010) but this has not been consistent (Halawaty et al., 2010; Skalba et al., 2011, La
258 Marca et al., 2012; Overbeek et al., 2012). In a recent study, AMH was negatively related to BMI but the
259 relationship was age-dependent (La Marca, et al., 2012) suggesting that the relationship is secondary to
260 the stronger relationship of the two variables with age. Contradictory result have also been reported on
261 the relationship between smoking and AMH, with some studies reporting reduced AMH levels in
262 smokers (Freeman, et al., 2007, Plante et al., 2010, Freour et al., 2012) and others reporting similar
263 values (Nardo et al., 2007; Dafopoulos et al., 2010; Waylen et al., 2010; La Marca, et al., 2012).

264 Analysis of intra-individual variability may be secondary to true biological variations in AMH levels in the
265 circulation. The inter-menstrual cycle variability has been appropriately analyzed in two prospective
266 studies (Fanchin et al., 2005, van Disseldorp et al., 2010), both of which concluded that 89% of the
267 variation in AMH was due to between-subject variation, while only 11% of variability was secondary to
268 individual fluctuation in AMH levels. Both studies found a similar intra-class coefficient (ICC) of 0.89,
269 which is the ratio of the inter-individual variability over the total variability thus the higher the ICC, the
270 lower the intra-individual variability. The majority of studies indicate that AMH is relatively stable
271 through the menstrual cycle, as would be expected since the dominant follicle and corpus luteum do not
272 secrete AMH (Hehenkamp, 2006; La Marca et al., 2006; Tsepelidis et al., 2007) (Figure 3). Van
273 Disseldorp, et al. (2010) calculated the intra-individual CV of AMH to be 13 %, with intra-individual
274 fluctuations within the same quintile in 72% of women and to cross two quintiles in only 1%. In contrast,
275 a recent but small study found a reduction in circulating AMH in the luteal phase and intra-individual
276 variance of AMH to be as high as 80% (Hadlow et al., 2013). In a prospective study based on 20 women,
277 the authors described two different patterns for AMH dynamics throughout the menstrual cycle. The
278 “younger ovary” pattern had higher mean AMH and significant variations in AMH levels throughout the
279 cycle. This was in contrast with an “aging ovary” pattern with low mean AMH, shorter menstrual cycle

280 lengths, and very low variation in AMH levels, suggesting diminished ovarian reserve. Fluctuations were
281 randomly distributed during the cycle indicating that measuring on a fixed day would not be
282 advantageous.

283 The literature also contains contradictory reports regarding the influence of conditions associated with
284 gonadotrophin suppression, particularly hormonal oral contraception use and pregnancy, with serum
285 AMH level. It seems likely that weak study size and design underlies this confusion. Recently a cohort
286 study based on 863 women (228 OC-users and 504 non-users) reported that AMH serum levels were
287 29.8% lower in oral contraception users than controls (Bentzen et al., 2012). This has been recently
288 confirmed by Dolleman et al. (2013). In a small but randomized trial of 42 healthy women administered
289 oral, transdermal or vaginal ring hormonal contraception for 9 weeks, AMH levels decreased by almost
290 50% in all treatment groups (Kallio et al., 2013). Conversely, serum AMH level increases in subsequent
291 natural cycles after stopping with hormonal contraception (van den Berg et al., 2010). Similarly in
292 relation to pregnancy, in the only longitudinal study available (n=60) a significant decrease in AMH levels
293 was found in the 2nd and 3rd trimesters compared to the 1st trimester, with a mean reduction at the end
294 of pregnancy of about 50% (Nelson et al., 2010). Such a decline in AMH levels during pregnancy has
295 been recently confirmed by Köninger et al. (2013) in a cross-sectional study. While this no doubt reflects
296 reduced follicular maturation, there may also be a contribution of pregnancy-associated haemodilution
297 and increased plasma-protein binding.

298 In conclusion, fluctuations in AMH levels have been reported for a number of conditions and this has to
299 be taken into account when interpreting values in clinical practice. While fluctuations in the menstrual
300 cycle appear to be random and minor hence permitting the measurement of AMH independently of the
301 cycle phase, ovarian suppression as induced by physiological or pharmacological interventions may
302 reduce AMH levels. Thus, serum AMH may not retain its accuracy as a predictor of the ovarian reserve in
303 women using long-term hormonal contraception.

304

305 **Derivation of a normative model for AMH from conception to menopause**

306 The emerging value of AMH measurement requires understanding of its pattern across the whole
307 female life-course. Most published studies that report AMH in normal girls and women include only a
308 relatively small age range, thus a 'data-driven' approach has been used (Kelsey et al., 2012). This
309 involved extracting data using a semi-automated procedure, and combined it with other unpublished
310 data. The resulting combined dataset (n = 3,260; age range -0.3 years to 54 years)(Kelsey et al., 2011)
311 forms a representative sample of AMH levels in the population of healthy female humans, and can
312 therefore be used as a basis for a predictive model of serum AMH level with changing age and was used
313 to generate and validate the model.

314 Analysis of the model shows that the dynamics of circulating AMH levels throughout life can be split into
315 several distinct phases (Figure 4). A peak shortly after birth confirms that girls also undergo a ‘mini
316 puberty’ of the neonate, following which there is a sustained rise to about 9 years of age. There is an
317 inflection with even a slight decline during the pubertal ages (9–15 years), followed by a second growth
318 phase to a peak at an age of about 25 years. After this, there is a steady decline to undetectable levels at
319 an average age of 50–51 years, corresponding to the menopause.

320 When non-growing follicle (NGF) recruitment dynamics are considered and compared to AMH levels
321 (Figure 4) there is a strong and positive correlation ($r = 0.96$) between declining AMH and declining
322 numbers of recruited NGFs after age 25 (the average age of peak AMH). This observation underpins the
323 use of serum AMH level as an indirect indicator of human ovarian reserve for ages after the mid-
324 twenties. Before the age of 25, the relationships between AMH and ovarian reserve are more complex
325 with overall a positive relationship between rising AMH and increasing follicle growth activation, and
326 thus we would recommend caution in the interpretation of AMH concentrations in girls and young
327 women as an indirect indicator of ovarian reserve.

328 **Ovarian Reserve assessment**

329 **Assessment of Ovarian Reserve in normal women**

330 From the ART literature, it is clear that AMH can predict the ovarian response to hyperstimulation (Broer
331 et al., 2013). AMH is superior to female age in assessing the quantitative aspects of the ovarian reserve
332 but its value is much more limited in the prediction of ongoing pregnancy. Indeed no combination of
333 ovarian reserve tests (ORTs) has been able to improve the accuracy of female age in identifying those
334 with a close to zero prognosis (Hendriks et al., 2008, Broer, et al., 2013). Qualitative aspects of the
335 ovarian reserve are much more difficult to capture.

336 The role for AMH as a predictor of natural fertility has been studied in a limited number of papers. In a
337 prospective study of women mostly in their 30’s, those with low AMH had significantly reduced
338 fecundability, after adjustment for age (Steiner, et al., 2010). In contrast, fecundability in healthy young
339 women with no prior knowledge of their fecundity, appeared not to be compromised if very low AMH
340 levels were present (Hagen et al., 2012). However, it must be stressed that these results were obtained
341 with the Gen II assay that provided at that time lower measurement than it was believed (see “assay”
342 section). Conversely, the probability of conceiving was reduced in women with high AMH levels,
343 suggesting that this represented women with overt or mitigated conditions of anovulation. Being a
344 quantity marker, the true value for AMH may therefore be found in predicting the timelines in the
345 ovarian ageing process that are dictated by quantity alone.

346

347 To study the value of the ORTs in the assessment of the future ovarian reserve status, long term follow
348 up studies are required, where several factors assessed at initiation of the follow up are linked to the
349 final outcome age at menopause. As menopause has a fixed time relation to earlier events such as onset
350 of cycle irregularity (average age 46 years) and the loss of natural fertility (average age 41 years), a
351 woman's reproductive lifespan can be predicted from forecasting age at menopause. To date, a total of
352 four datasets are available addressing this issue. In two small studies, it has been demonstrated that
353 across a period of 9 and 12 years, AMH level will adjust the predictions that can be based on female age
354 at the moment of AMH sampling, so that women with low age-specific AMH will have menopause
355 earlier and vice versa (Tehrani et al., 2009, Broer et al., 2011). A larger analysis is now available from the
356 Iranian study (Tehrani et al., 2013). A third study confirmed these findings in a group of women of late
357 reproductive age, but with still detectable levels of AMH (Freeman, et al., 2007). All these datasets
358 however have very wide confidence intervals in the predictive value of a single AMH measurement. The
359 rate of change over time may also affect the time to menopause, and be susceptible to extrinsic as well
360 as intrinsic factors.

361 Genetic factors have proven to play a major role in determining the variation in menopausal age,
362 as demonstrated in several mother-daughter, twin and sib-pair studies. Next to genetic factors, several
363 environmental and life-style factors like smoking, body mass index, use of alcohol and parity have
364 claimed to influence menopausal timing as well. Thus, menopausal age is considered a complex genetic
365 trait. From a recent review (Voorhuis et al., 2010), it became apparent that a number of genetic regions
366 and variants involved in several possible pathways underlying timing of age at menopause could be
367 identified. Regarding a potential role for AMH or its receptor in modulating the rate of follicle loss from
368 the primordial follicle pool, it has been demonstrated in two separate studies that common variation in
369 the AMHR2 gene modifies the relationship between parity and age at natural menopause (Kevenaer et
370 al., 2007; Voorhuis et al., 2010). Moreover, interactions between common variation in the AMH and
371 AMH receptor II gene in their effect on menopause have further supported a potential role for factors
372 that steer initial follicle recruitment (Braem et al., 2013).

373 The value of predicting age at menopause serves multiple targets. First of all, the ability to assess the
374 future ovarian reserve status, and thereby the reproductive lifespan of an individual women, will have
375 implications for female infertility. Because of the fixed time interval that is believed to be present,
376 prediction of age at menopause will predict the age of natural end of fertility. If such predictions could
377 be made early in life, with sufficient accuracy, this could have a great influence on individual women
378 making decisions regarding career and a wish to have children. It is at present unclear whether AMH
379 measurement meets those criteria.

380 **AMH in the assessment of ovarian damage from chemotherapy, radiotherapy and surgery.**

381

382 The relationship between serum AMH and the number of small growing and indeed primordial follicles
383 has made it a prime potential tool for the investigation of gonadotoxicity of cancer therapy and of loss
384 of the ovarian reserve from ovarian surgery. AMH offers the possibility of a more accurate assessment,
385 revealing partial loss of the ovarian reserve, as well as ovarian failure. It may also be of value in children
386 where FSH and inhibin B are not useful, and in individualising the degree of damage when measured
387 prospectively.

388 A decrease in serum AMH was first described in women who had had childhood cancer but who still had
389 regular menses, compared to an age matched control group (Bath et al., 2003). In contrast there was no
390 difference in serum FSH or inhibin B between groups. Similar findings have been shown in breast cancer
391 survivors (Partridge et al., 2010). AMH was decreased in a study of ovarian function in young adults
392 following treatment for childhood Hodgkin lymphoma with a clear dose response demonstrated
393 between the number of chemotherapy cycles and the serum AMH (van Beek et al., 2007). FSH also rose
394 with increasing treatment, but AMH appeared to have greater sensitivity to detect ovarian damage at
395 lower doses of chemotherapy. The gonadotoxicity of alkylating agent based protocols has been shown in
396 a range of childhood and adult malignancies (Rosendahl et al., 2008; Lie Fong et al., 2009; Gracia et al.,
397 2012) but is most clearly demonstrated in a prospective study in young women with lymphoma
398 (Decanter et al., 2010): AMH concentrations fell in all women during therapy but in the non-alkylating
399 agent group there was then recovery to concentrations similar to pre-treatment whereas there was no
400 evidence of recovery in women treated with alkylating agent based therapies.

401 Radiotherapy is also widely recognised to cause ovarian damage even at low doses and women treated
402 with radiotherapy that includes the pelvis (including abdominal pelvic therapy in children or total body
403 irradiation) generally have very low or undetectable AMH concentrations (Gracia, et al., 2012) (Lie Fong,
404 et al., 2009).

405 Most of these studies were retrospective in nature, with no pre-treatment samples taken. There is also a
406 dearth of data linking post treatment AMH to other clinical variables, most importantly fertility and
407 subsequent reproductive lifespan, although a recent analysis shows a high prevalence of successful
408 pregnancy in childhood lymphoma survivors despite low AMH concentrations (Hamre et al., 2012). A
409 prospective study in women with newly diagnosed breast cancer linked pre-treatment AMH with long
410 term ovarian function at 5 years (Anderson and Cameron, 2011), pre-treatment serum AMH being
411 markedly higher in women who continued to have menses. The predictive value of AMH for post-
412 chemotherapy ovarian function has subsequently been confirmed (Anderson et al., 2013) allowing the
413 development of prediction tools combining age and AMH (Figure 5). It therefore appears that in addition
414 to reflecting post-chemotherapy (or radiotherapy) damage, AMH is also able to predict on-going ovarian
415 activity after such treatment, and the existing data suggest it is likely to be more robust than either FSH
416 or inhibin B in this regard. Consistent with this, a study in younger women has demonstrated that
417 pretreatment AMH predicts post-chemotherapy recovery, with a more rapid recovery in women with

418 higher pretreatment AMH (Dillon et al., 2013). Older women with cancer may have lowered
419 pretreatment AMH concentrations; this was not observed in younger women (Su et al., 2013).
420 Substantial prospective studies are required to develop a clearer analysis of the predictive value of AMH
421 in different circumstances and it may be of value in information provision for example regarding the
422 need for fertility preservation strategies.

423 AMH is detectable in girls of all ages, unlike other reproductive hormones, and rises steadily through
424 childhood thus may be of value in the assessment of ovarian function in pre-pubertal girls. In a
425 prospective analysis of girls with varied diagnosis (and therefore undergoing differed therapies) at
426 different ages, AMH declined during repeated chemotherapy cycles (Brougham et al., 2012). Strikingly,
427 in girls judged to be at medium or low risk of long-term ovarian damage, AMH recovered to
428 concentrations similar to pre-treatment, whereas in girls judged to be at high risk, serum AMH at the
429 end of treatment was undetectable and showed no evidence of recovery. Post-treatment AMH
430 therefore appeared to identify even very young girls who are very likely to require pubertal induction,
431 distinct from others who may be able to be reassured as to the likelihood of satisfactory ovarian
432 function later in life. Long term follow up of these different groups is required to ascertain fully the value
433 of post childhood cancer AMH in predicting long term ovarian function whether reflected in achieving
434 spontaneous puberty, fertility or reproductive lifespan.

435 The impact of ovarian surgery on the ovarian reserve as measured by AMH has also been investigated,
436 and two systematic reviews of the impact of ovarian surgery for endometriosis have been published
437 (Raffi et al., 2012; Somigliana et al., 2012). Both analyses highlight the heterogeneity of study design and
438 the difficulty in pooling data. However both conclude that ovarian endometrioma surgery is associated
439 with a decline in serum AMH, indicating the removal of a significant part of the ovarian reserve. A
440 subsequent large retrospective analysis has confirmed the impact of endometrioma surgery on the
441 ovarian reserve as detected by serum AMH (Streuli et al., 2012), and these findings should be taken in to
442 account in the planning and decision making process relating to ovarian surgery in women desirous of
443 future pregnancy.

444 **Assessment of Ovarian Reserve in infertility and ART patients**

445 Age and ovarian reserve are potentially the most important patient characteristics determining the
446 success of assisted conception, with interpretation of AMH in an age-specific manner now feasible
447 (Nelson et al., 2011a; Nelson et al., 2011c; Almog, et al., 2011). Recognition of the linear relationship of
448 AMH with oocyte yield was a critical step forward (Nelson et al., 2007; La Marca et al., 2010). That AMH
449 can predict ovarian response accurately (Broer et al., 2011)(Broer et al., 2009) enables clinicians to avoid
450 iatrogenic complications and to choose the optimal stimulation strategy. This also ensures that patients
451 are counselled appropriately with realistic expectations of the outcome of their ovarian stimulation.

452 At one extreme of the response spectrum we can identify women who are at risk of OHSS (Al-Inany et
453 al., 2011, Broer, et al., 2011). We can adjust our stimulation strategy to incorporate GnRH antagonists
454 (Al-Inany, et al., 2011) reducing the risk of this potentially fatal complication (Acolet et al., 2005, Braat et
455 al., 2010). Choosing a GnRH antagonist protocol and adjusting the FSH dose according to a high serum
456 AMH level should preclude OHSS but at present, however, only locally-derived thresholds can be used
457 since there is no consensus on an universal threshold (Broer, et al., 2011). This approach has particular
458 benefits for women undergoing altruistic oocyte donation, removing much of the integral risk of IVF
459 (Bodri et al., 2009). Conversely maximising follicular recruitment would seem appropriate if a poor
460 response was anticipated, although the optimal strategy for the poor responder remains debated
461 (Ferraretti et al., 2011). At present the value of a mixed strategy in an ART programme has yet to be fully
462 elucidated, but for centres where agonist strategies still dominate the advantage of an AMH-based
463 approach over conventional dose adjustment and long course agonist for all has been demonstrated
464 (Nelson et al., 2009).

465 The ability to predict a very poor response has resulted in some centres withholding the first treatment
466 cycle if a very low AMH is detected, with an overall improvement in results of the programme and
467 substantial cost savings (Yates et al., 2011). However even women with AMH concentrations at the limit
468 of assay sensitivity have a significant chance of conception through IVF, thus this approach appears
469 unjustified (Anderson et al., 2012). Inevitably this chance will be lower than for a woman of the same
470 age with a higher ovarian reserve (La Marca, et al., 2010) but to withhold treatment and not actually
471 confirm a predicted poor response at present purely based on an AMH would seem inappropriate. This
472 is particularly the case as this approach has not been incorporated into cost-effectiveness models with
473 other more accurate population level models available (Lawlor and Nelson, 2012; Nelson and Lawlor,
474 2011).

475 Whether knowing the anticipated oocyte response has a beneficial psychological effect for the couple
476 and thereby reduces cycle drop out has not been formally evaluated. Discussion of the ovarian
477 assessment report may set patient's expectations appropriately particularly at the bottom end of the
478 spectrum where only a few oocytes may be retrieved. Given that many women do not fully appreciate
479 the detrimental effect of age on oocyte number, the ability to guide them on overall success using a
480 combination of their age as a surrogate for oocyte quality, and AMH for oocyte yield is a powerful tool
481 (La Marca et al., 2011).

482 It is likely in the future that with standardisation of AMH measurement and stimulation strategies,
483 multivariate prediction models with tight confidence intervals will be able to be created and
484 individualised reports generated. Steps on this path have already been made with optimal prediction of
485 excessive response achieved by combining age, AMH and antral follicle count (Broer et al., 2011a) and
486 refinement of gonadotropin dosing by combining AMH with FSH and age (La Marca et al., 2012). The

487 future is therefore likely to harness the collective power of biomarkers including AMH to ensure true
488 personalisation of ovarian stimulation.

489

490 **Factors influencing the relationship between and the predictability of AMH and antral follicle count**
491 **(AFC)**

492 The follicular pool that influences serum AMH levels the most probably is that of 1-2 mm follicles,
493 although some analyses have suggested a slightly larger size (Jeppesen, et al., 2013) (see section 3-1).
494 This notion assumes a particular importance not only when we analyze the strength of the relationship
495 between the ultrasonographic counting of antral follicles (AFC) and serum AMH levels but also when we
496 compare the clinical predictability of both parameters.

497 Although the positive relationship between AFC and serum AMH levels has been recognized for over ten
498 years (Fanchin et al., 2003), cases of discrepancy are sporadically observed (Schipper et al., 2012). These
499 cases may result, at least in part, from technical difficulties but other physiological contingencies may
500 influence this expected relationship. According to recent guidelines (Broekmans et al., 2010) and current
501 clinical practice worldwide, ultrasonographic counting considers antral follicles whose diameter varies
502 considerably, from 2 to 10 mm. It is also noteworthy that ultrasound technology cannot distinguish
503 healthy from atretic follicles. Therefore, the strength of the correlation between AFC and serum AMH is
504 influenced by at least 2 additional factors. The first is antral follicle sizes. It is likely that a patient whose
505 AFC is mostly represented by small follicles (1-2mm) will display higher serum AMH levels than a patient
506 who has a majority of large antral follicles (>6 mm). The second factor is follicle "health" as granulosa
507 cell atresia may hinder AMH production. Further clinical studies are needed to confirm these
508 hypotheses.

509 In line with this, both AMH and AFC have been shown to be useful markers of the ovarian response to
510 controlled ovarian hyperstimulation (Broer, et al., 2013). Again here, two other refinements should be
511 brought to this clinical observation. On the one hand, it is probable that, in the beginning of the follicular
512 phase, it is the large antral follicles that will respond first to gonadotropin treatment. As these follicles
513 are already losing their ability to produce AMH, AFC might better predict ovarian response than AMH
514 (Mutlu et al., 2013). On the other hand, if we consider that atretic antral follicles will not properly
515 respond to exogenous FSH, AMH should be the most reliable marker as it is not produced by atretic
516 follicles that still are counted by ultrasound. Another pertinent issue regarding both biomarkers is that,
517 contrary to AFC, AMH is also an important regulator of ovarian function, as discussed above. In the
518 ovary, AMH exerts an inhibiting role on many follicular functions, including granulosa cell sensitivity to
519 FSH. In support of this, antral follicle responsiveness to exogenous gonadotropins, clinically assessed by
520 the Follicle Output RaTe (FORT), is inversely correlated with serum AMH (Genro et al., 2011).

521 Therefore, from a clinical standpoint, both AMH and AFC provide the physician with useful information
522 regarding ovarian follicular status and responsiveness to controlled ovarian hyperstimulation. While
523 AMH provides information essentially on the number of very small, non-atretic follicles, AFC is
524 contributive to detect follicle sizes and evaluate size discrepancies, with both analyses being
525 complementary to the proper adaptation of the type of stimulation required by the patient.

526 **Polycystic Ovary Syndrome (PCOS)**

527 **AMH and its putative role in PCOS pathophysiology**

528 PCOS, a heterogeneous condition, is the most prevalent endocrine disorder in women, affecting 5 to
529 10% of the female population (Franks, 2008). Women with PCOS present with a range of symptoms
530 such as acne, hirsutism and/or menstrual irregularities and have an increased risk of type II diabetes.
531 The condition imposes a considerable economic burden on health systems internationally (Azziz et al.,
532 2005). Polycystic ovaries (PCOs) are characterised by an increase in the number of follicles at all growing
533 stages (Hughesdon, 1982; Webber et al., 2003; Maciel et al., 2004). PCOS is almost certainly a genetic
534 condition (Kosova and Urbanek, 2013), but the cause of the change in ovarian and the cause of
535 anovulation which affects a subgroup of these women remains unknown.

536 The ability of AMH to alter early follicle growth was demonstrated by the AMH knock-out mouse model
537 (Durlinger, et al., 1999, Durlinger, et al., 2002) in which there is an increase in the initiation of primordial
538 follicles into the growing pool (see section 3). This morphology appeared similar to that seen in
539 polycystic ovaries (PCOs) and so an assessment of the production of AMH by PCOs was carried out.
540 Stubbs et al., 2005 found fewer primordial and transitional follicles positively stained for AMH from
541 anovulatory PCO than in normal ovaries. Reduced AMH in anovulatory PCO might enhance the transition
542 of follicles to the growing phases, or might be a marker of abnormal early follicle growth in PCOS.

543 Serum AMH is two to four-fold higher in women with PCOS than in normal women (Pigny et al., 2003;
544 Laven et al., 2004; Park et al., 2010; Lie Fong et al., 2011). This increase in serum AMH was thought to
545 reflect the increased number of small antral follicles in which AMH production is highest. However,
546 when production of AMH per granulosa cell was compared between normal ovaries, ovulatory and
547 anovulatory PCOs (Pellatt et al., 2007), AMH production was on average 75 times higher per granulosa
548 cell from anovulatory PCOs and 20 times higher from ovulatory PCOs. This indicates that the increase in
549 AMH is due to an intrinsic property of granulosa cells in PCOs, a property that persists even after
550 stimulation for IVF (Catteau-Jonard et al., 2008). These increased concentrations are also found in
551 follicular fluid (Das et al., 2008).

552 The cause of such high levels of AMH in antral follicles in PCOS is currently unknown. However there is
553 evidence to support a role for androgens as a positive correlation with AMH in serum has been reported

554 (Carlsen et al., 2009; Pigny, et al., 2003; Laven, et al., 2004; Eldar-Geva et al., 2005), and over-production
555 of androgens is an intrinsic defect of theca from PCOs (Gilling-Smith et al., 1994). It is curious that AMH
556 should be lower in preantral follicles and then higher once the follicle reaches the antral stage, however
557 prenatal testosterone treatment of sheep produced precisely this effect (Veiga-Lopez et al., 2011). In
558 vitro however, androgens have not been shown to do this and indeed androgens have been shown to
559 reduce antral follicle granulosa cell AMH production in a bovine model (Crisosto et al., 2009). In human,
560 serum AMH levels decrease in female to male transsexual women using testosterone as cross-sex
561 therapy (Caanen M et al., 2013). Other groups have demonstrated inhibition of AMH production by
562 gonadotrophins, particularly FSH (Baarends et al., 1995; Panidis et al., 2011). Others found no such
563 inhibitory effect on granulosa cells from normal ovaries; in contrast, FSH did inhibit AMH production in
564 cultured granulosa cells from polycystic ovaries (Pellatt, et al., 2007) whereas LH significantly stimulated
565 production.

566 Although many aspects of AMH action in the ovary remain to be elucidated, knowledge is emerging.
567 AMH significantly decreases FSH- and LH- induced aromatase expression in granulosa cells as well as
568 reducing the activity of the ovary-specific aromatase promoter II (see section 3). This results in a
569 significant reduction in estradiol production (Pellatt et al., 2011). AMH also inhibits FSH-stimulated FSH
570 receptor mRNA expression (Pellatt, et al., 2011). The fact that AMH is inhibitory of factors required for
571 follicle growth adds considerable significance to the finding of high AMH in PCOS. LH reduces AMHR11
572 expression in granulosa luteal cells collected from women with normal ovaries and ovulatory PCOS, but
573 was unable to do so in women with anovulatory PCOS (Pierre et al., 2013). It can be envisaged that AMH
574 content in antral follicles in these ovaries would be sufficient to inhibit FSH-stimulated aromatase
575 expression and would thus prevent the inhibitory effect of estradiol on AMH production (Figure 1). This
576 effect would be amplified by the loss of LH-induced down-regulation of AMHR11 expression in women
577 with anovulatory PCOS. These findings suggest that AMH may contribute to anovulation in PCOS. In
578 agreement, it has been shown that emergence of a dominant follicle in anovulatory women with PCOS
579 under recFSH is preceded by a significant reduction in serum AMH level (Catteau-Jonard et al., 2007).

580 **AMH in diagnosing PCOS: a shift from ultrasound to laboratory**

581 Given its strong involvement in the pathophysiology of PCOS (see section 5.1), serum AMH is a subject
582 of special interest for clinicians involved in this field. There is considerable interest in whether it might
583 become part of the diagnostic criteria for the condition, although this is at present premature. It may
584 also shed light on different subtypes of this diverse condition leading to greater understanding of the
585 disordered follicle growth. Certainly, the serum AMH concentration appears to be greatly increased in
586 most patients with PCOS (Pigny, et al., 2003, Laven, et al., 2004, Li et al., 2011). This elevation is highly
587 pertinent as it has been shown that polycystic ovaries (PCO) exhibit an increased number of AMH-
588 producing pre-antral and small antral follicles, the latter expressing the most AMH (Weenen, et al.,

589 2004) and contributing the most to the circulating AMH (Jeppesen, et al., 2013). In addition, production
590 of AMH is greatly increased in GC from PCO, especially if the patient is oligo-anovulatory, as discussed
591 above (Pellatt, et al., 2010). Therefore, not surprisingly, many authors have reported a strong correlation
592 between plasma levels of AMH and follicle count on ultrasound in PCOS patients. The strength of this
593 relationship is even greater with newer ultrasound technology allowing the counting of 1-2 mm follicles
594 (Dewailly et al., 2011).

595 The strong association between AMH and follicle count has led some authors to compare the
596 performance of one against the other for the diagnosis of PCOS. However, the results in the current
597 literature are not homogeneous between studies, as well demonstrated in a recent compilation
598 (Iliodromiti et al., 2013). Part of this heterogeneity is due to the lack of well-defined populations. In
599 particular, it must be stressed that many authors have used the threshold for follicle excess that was
600 established in 2003 at the Rotterdam Consensus Conference to define PCOM (Balen et al., 2003), namely
601 12 follicles of 2-9 mm diameter per ovary. With the latest generation of ultrasound equipment and using
602 well-defined populations, recent studies have proposed to increase this threshold to 19 or 25 (Dewailly,
603 et al., 2011, Lujan et al., 2013, respectively). This threshold will probably continue to evolve in parallel
604 with the technical improvement of ultrasound equipment.

605 Beside the flaw in the ultrasound definition of controls and patients, the variability of the results can
606 also be explained by the problem that prevails with serum AMH assays. About half of the previous
607 studies were performed using either the DSL or IOT assays (Iliodromiti, et al., 2013), for which
608 concordance in the values is problematic (see above). More recent studies using the Gen II kit should
609 also be interpreted with caution (see above).

610 It is therefore impossible to date to propose a consensual and universal diagnostic threshold for serum
611 AMH that is predictive of PCOS. Using the IOT assay, serum AMH was found to be more efficient than
612 the follicle count with excellent sensitivity and specificity for a threshold of 35 pmol/l (4.9 ng/ml)
613 (Dewailly, et al., 2011). Contrary to other studies, specific thresholds for AMH and follicle count were
614 calculated without using pre-determined values. In addition, women with supposedly asymptomatic
615 PCOM were excluded from the control group of regularly menstruating women by cluster analysis. If
616 these results can be replicated with the new AMH assays, serum AMH may become an accurate and
617 reliable marker that may eventually replace the follicle count which itself, in turn, suffers from great
618 controversy in the current literature. It is reasonable to propose that the increased serum AMH is a
619 surrogate to the term "PCOM" in the Rotterdam classification (Rotterdam ESHRE/ASRM-sponsored PCOS
620 consensus workshop group, 2004). Further, since we have now at our disposal two different markers,
621 one being morphological (PCOM) and the other being biochemical (increased serum AMH), the terms
622 "PCO-like abnormalities" (PCO-L) may become more accepted as the third item of the Rotterdam
623 classification (Robin et al., 2012).

624 In addition, the serum AMH correlates with the severity of PCOS and precisely with the severity of both
625 hyperandrogenism (Piouka et al., 2009) and oligo-anovulation (Laven, et al., 2004, Catteau-Jonard et
626 al., 2012). By principal component analysis, it has been shown that a high serum AMH level can be
627 considered a marker of hyperandrogenism and may therefore also be considered as a replacement for
628 this other item in the Rotterdam classification (Dewailly et al., 2010). This would reconcile the different
629 classifications currently available for the diagnosis of PCOS since some of them necessarily require the
630 presence of hyperandrogenism to retain the diagnosis (Azziz et al., 2009). The only exception to this
631 assertion would be the presence of PCOS in women with type 1 diabetes, where serum AMH does not
632 correlate to androgen levels (Codner et al., 2007).

633 Therefore, to establish the diagnosis of PCOS, after exclusion of other diagnoses, oligo-anovulation and
634 HA should first be required. In the cases where one is missing, then “PCO-L” (i.e., high AFC and/or serum
635 AMH level) could be used as a surrogate for either oligo-anovulation or HA. It must be stressed,
636 however, that the thresholds for an excessive AFC and serum AMH level have to be revisited and
637 validated worldwide in populations of different ethnicity. Meanwhile, local in-house control data can be
638 used. We think this information is important and useful for diagnostic concerns as well as for
639 phenotype/genotype analysis within genetic studies.

640 The diagnostic value of serum AMH concentrations has also been studied in adolescents since
641 ultrasound is often unreliable in detecting PCOM in this population. A study in Chilean adolescents
642 identified a cut-off serum AMH concentration of 60 pmol/l (with the IOT assay) to diagnose PCOM in
643 regularly menstruating adolescents, with a sensitivity and specificity of 64% and 90% (area under the
644 ROC curve = 0.87) (Villaruel et al., 2011). The results were not as good in Australian adolescents with
645 the same assay (area under the ROC curve = 0.67) leading the authors to conclude that serum AMH was
646 a questionable surrogate for PCOM in adolescents (Hart et al., 2010).

647 Finally, in addition to its diagnostic role, the determination of AMH could be used in the future to
648 establish treatment protocols, and in particular to define the strategy for the induction of ovulation in
649 infertile oligo-anovulatory PCOS women. To date, there are very few studies that have examined the
650 predictive power of AMH assay for response to clomifene, recombinant FSH or to ovarian drilling.
651 Similarly, AMH is of value as a good predictor of the risk of ovarian hyperstimulation in an IVF setting
652 (Broer, et al., 2011) .

653 The current technical difficulties with the determination of serum AMH may have dampened the
654 enthusiasm of some clinicians for this marker of PCOM. However there are sufficient data to support the
655 view that this assay may replace (or be an alternative for) AFC in the Rotterdam classification, which will
656 make it even more reliable and more flexible, especially in situations when ultrasound is uninformative
657 or impossible, as in obese women or adolescents.

658

659 **Future avenues**

660 Recent years have shown multiple ways in which AMH is not only a “male” hormone but is emerging as
661 an invaluable tool offering new insights into ovarian function in childhood, adolescence and through the
662 reproductive years. Although knowledge of its precise roles in ovarian physiology still requires extensive
663 fundamental and clinical studies, it is already clear that AMH is crucial in maintaining the right tempo of
664 folliculogenesis in the ovary (although there are only very limited human data), making it one of the
665 most important ovarian hormones and one of the most crucial factors underpinning female fertility.
666 Whether its action is exclusively intra-ovarian, within and between follicles, is a challenging issue for
667 future research. We should think about possible endocrine effects of this hormone, possibly in ovary-to-
668 ovary interaction or in hypothalamic-pituitary-ovarian integration.

669 At the current time, the clinical use of serum AMH assay is hampered by technical issues undermining its
670 reliability. It is likely that these issues will be rapidly solved and the advent of more sensitive assays may
671 confirm that serum AMH level is the best biochemical marker of ovarian function in a large array of
672 clinical situations, both in childhood and adulthood. For the first time in female reproductive biology, we
673 have at our disposition an easy measure of the submerged part of the iceberg of follicle growth, i.e., the
674 intrinsic so-called “acyclic” ovarian activity.

675

676

677 **Author's Roles**

678 This paper is a summary of the presentations at the ESHRE campus workshop on AMH in Lille, France, on
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682

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Legends to figures

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1194 **Figure 1: Schematic model of anti-Müllerian hormone (AMH) actions in the ovary.**

1195 AMH, produced by the granulosa cells of small growing follicles, inhibits initial follicle recruitment and
1196 FSH-dependent growth and selection of preantral and small antral follicles. In addition, AMH remains
1197 highly expressed in cumulus cells of mature follicles. The inset shows in more detail the inhibitory effect
1198 of AMH on FSH-induced *CYP19a1* expression leading to reduced E2 levels, and the inhibitory effect of E2
1199 itself on AMH expression. AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone; T,
1200 testosterone; E2, estradiol; Cyp19a1, aromatase. Figure modified from van Houten et al. (2010).

1201

1202 **Figure 2: Schematic presentation depicting the processing of AMH.**

1203 AMH is produced as a precursor protein consisting of disulphide-linked monomers. Upon cleavage by
1204 prohormone convertases the protein is cleaved into pro- and mature homodimers, which remain non-
1205 covalently associated. AMH enzyme-linked immunosorbent assays (ELISA) have been developed to
1206 detect AMH in circulation. The regions that are recognized by the monoclonal antibodies used in the
1207 ultrasensitive IOT assay and the Gen II assay (previously DSL) are indicated. For the Gen II assay, the
1208 capture antibody recognized the mature region and the detector antibody recognizes the proregion.

1209

1210 **Figure 3: AMH variability throughout the menstrual cycle. Serum AMH appears to be stable.**

1211 *(Reproduced with permission from (a) La Marca et al., 2006, (b) Hehenkamp et al., 2006 and (c)*
1212 *Tsepelidis et al., 2007).*

1213

1214 **Figure 4: AMH and follicular recruitment profile across the lifespan.**

1215 Comparison of serum AMH concentrations with NGF recruitment rates. The red line is the log-
1216 unadjusted validated AMH model (Kelsey et al., 2011), peaking at 24.5 years. The blue line denotes the
1217 numbers of NGFs recruited per month towards the maturation population (Wallace and Kelsey, 2010),
1218 with peak numbers lost at age 14.2 years on average. Correlation coefficients (*r*) are given for AMH
1219 concentrations against follicular recruitment for each developmental phase; from birth to puberty (age 9
1220 years), during puberty (9 – 15 years), post-puberty (15- 25 years) and mature adults (>25 years).

1221

1222 **Figure 5: Classification mosaic chart for ongoing menses (M) or chemotherapy-related amenorrhea (A)**
1223 **using pre-chemotherapy serum AMH and chronological age as predictor variables, in women with**
1224 **early breast cancer.**

1225 The primary cutoff values are both for AMH, with below 3.8 pmol/L predicting amenorrhea and above
1226 20.3 pmol/L predicting ongoing menses. Between these AMH levels there is an age threshold at 38.6
1227 years, above which amenorrhea is predicted and below which ongoing menses are predicted. The

1228 classification schema has sensitivity 98.2% and specificity 80.0%. Reprinted with permission from
1229 Anderson et al 2013, Eur J Cancer.

1230

1231 **Figure 6: rationale for the use of serum AMH assay as a probe for PCOM**

1232 (A) All growing follicles secrete AMH but serum AMH reflects only the secretion from bigger follicles that
1233 are in contact with the vascular bed. As the numbers of follicles in all growth stages are strongly related
1234 to each other, serum AMH is considered to reflect the sum of growing follicles but not the number of
1235 primordial follicles that do not secrete AMH (see section 3-1).

1236 (B) In PCO, the numbers of all growing follicles is increased, resulting in a marked increase in serum AMH
1237 level (see section 5). This marker may be considered as a deeper and more sensitive probe to define
1238 follicle excess than the follicle count by ultrasound (U/S) since it appraises more follicle classes (blue
1239 arrows).

1240