

1 **Title:** Anti-Müllerian hormone in PCOS: a review informing international guidelines

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40 **Key words:** Polycystic Ovary Syndrome, review, Anti-Müllerian Hormone, adolescent, adult,
41 diagnostic accuracy

42 **Abstract**

43 Polycystic ovary syndrome (PCOS) affects 8-13% of women. Rotterdam diagnostic criteria
44 include polycystic ovarian morphology (PCOM) on ultrasound, yet given recognised
45 challenges, serum Anti-Müllerian Hormone (AMH) is proposed as an alternative. To inform
46 international PCOS guidelines, a systematic review was completed. Key identified gaps
47 include large international studies in well-defined populations across the life span,
48 clustering of AMH with PCOS features, relationships to long term health outcomes,
49 improved quality, assays standardisation and sample handling, all needed to determine cut-
50 offs. Here we identify research priorities to address these gaps and enhance AMH utility in
51 PCOS. Once issues are addressed, AMH levels could replace more costly and less accessible
52 ultrasound in PCOS diagnosis

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55 Challenges in ultrasound PCOM detection

56 Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women
57 of reproductive age with a reported prevalence of 8-13% [1-5]. The condition is
58 heterogeneous [6], and women may present with reproductive, endocrine, metabolic, and
59 psychosocial symptoms which vary across their lifespan [7]. The Rotterdam criteria require
60 that women fulfil two of the following three criteria to be diagnosed with PCOS: oligo- or
61 anovulation, clinical and/or biochemical signs of hyperandrogenism, and/or polycystic
62 ovaries on ultrasound [8-10], with the exclusion of other relevant disorders.

63

64 Within the diagnostic criteria, polycystic ovarian morphology (PCOM) on ultrasonography is
65 defined by either total ovarian volume or follicle number per ovary (FNPO). Original cut-offs
66 for PCOM were based on limited evidence [11] and were recently revised in the new
67 International PCOS guidelines, whilst also highlighting the controversy and challenges with
68 this criteria [1-4]. Determining FNPO is operator and equipment-dependent, limiting accuracy
69 and reproducibility. Equipment advances increase sensitivity and in turn FNPO counts [1-4,
70 12, 13]. Ultrasound involves expensive equipment and trained personnel, leading to
71 increasing costs and impacting on accessibility. The ultrasound approach (transabdominal or
72 transvaginal) impacts on accuracy, and in some women transvaginal ultrasound is
73 unacceptable or may be perceived as invasive. Multi-follicular appearance on ultrasound
74 overlaps with PCOM diagnostic cut offs especially in adolescents, whilst in older women with
75 PCOS cut off values might be considerably lower [11]. Recent international PCOS guidelines
76 now recommend against using ultrasound in PCOS diagnosis within 8 years of menarche and
77 called for greater accuracy in PCOS diagnostic criteria worldwide [1-4].

78 AMH as a potential alternative to ultrasound PCOM detection

79 AMH is a polypeptide of the transforming growth factor beta (TGF β) family, solely secreted
80 by granulosa cells of the pre-antral and small antral ovarian follicles [14]. AMH has been
81 shown in animal models of PCOS to have a possible causal role in development of the disorder
82 through in-utero exposure of the fetus to high AMH levels [15]. In women, AMH inhibits the
83 recruitment of primordial follicles out of the resting oocyte pool and may suppress follicle-
84 stimulating hormone (FSH) action contributing to ovulatory disturbances [16]. Overall, serum
85 AMH levels are significantly higher in women with PCOS compared with normal ovulatory
86 women [17, 18]. These data has led to the hypothesis that AMH could be a valuable surrogate
87 marker or an alternative to ultrasound FNPO count for detection of PCOM or in the overall
88 diagnosing of PCOS [16].

89 Recognised challenges in the use of AMH measurement in PCOS include variations across the
90 life span and problems with defining PCOM for comparison. AMH assays may also display a
91 differential response to pre-analytical proteolysis, conformational changes of the AMH dimer,
92 or the presence of interfering substances [19]. Appreciable sample-to-sample variability and
93 substantial discrepancies in between-assay conversion factors, suggests assay performance
94 issues. These issues were prioritised and addressed in the recent International evidence-
95 based guideline for the assessment and management of PCOS [1-4]. The aim of this systematic
96 review is to investigate whether AMH is effective for the detection of PCOM and/or diagnosis
97 of PCOS to inform international evidence based guidelines in PCOS.

98 Methods

99 This systematic review was conducted in accordance with the Preferred Reporting Items for
100 Systematic Reviews and Meta-Analyses (PRISMA) Statement [20] and was prepared to

101 inform recommendations in the updated and expanded evidence-based guideline for the
102 assessment and management of PCOS [4]. The methodology used for development of this
103 guideline is aligned with Australia's National Health and Medical Research Council (NHMRC)
104 [21], the European Society of Human Reproduction and Embryology (ESHRE) [22], and the
105 Grading of Recommendations, Assessment, Development and Evaluations (GRADE)
106 methodology [23], and is described in detail in the full guideline [4].

107 This systematic review addressed the evidence for the following two clinical questions:

108 1: Is AMH effective to diagnose PCOS?

109 2: Is AMH effective to detect PCOM?

110 *Systematic search for evidence*

111 A systematic search strategy was designed to identify the best available evidence to answer
112 the two clinical questions [24]. The search string comprised terms related to PCOS, PCOM,
113 diagnosis, and AMH, and was developed to retrieve articles addressing women with PCOS in
114 all cultural, geographical and socioeconomic backgrounds and settings. The search strategy
115 was limited to English language studies in humans, and there were no limits on year of
116 publication. A study design filter was not used.

117 *Selection criteria*

118 The Population of interest, Intervention, Comparison, and Outcome (PICO) framework was
119 used to guide the selection criteria for each clinical question presented in this systematic
120 review, and these were developed *a priori* by the multi-disciplinary guideline development
121 group [24]. These included reporting of results in the format of threshold, sensitivity,
122 specificity, area under the curve, and precision.

123 *Databases*

124 The following electronic databases were searched on June 26th 2017; Medline (Ovid)- Ovid
125 MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) and Ovid
126 OLDMEDLINE(R) 1950 to Present; EMBASE (Ovid); All EBM (Ovid)- including The Cochrane
127 Database of Systematic Reviews, DARE, CENTRAL and ACP Journal Club; PsycInfo (Ovid) and
128 CINAHL

129 *Evidence processing*

130 Studies were selected and appraised by one highly experienced reviewer (MM) in
131 consultation with colleagues using study selection criteria [24] established *a priori*. The
132 retrieved articles were first reviewed by title and abstract, and then full articles will be
133 retrieved for further assessment if the information given suggests that the study meets the
134 inclusion criteria.

135 *Assessment of methodological quality*

136 Methodological quality (i.e. risk of bias) of each of the included studies was assessed by one
137 reviewer for the adolescent studies (EB) and one reviewer for the adult studies (ECT), using
138 a critical appraisal template developed *a priori* [25]. Individual quality items were
139 investigated using a descriptive component approach that assessed attrition bias, reporting
140 bias, selection bias, performance bias, potential confounding, and appropriateness of the
141 statistical analysis. Any disagreement or uncertainty was resolved by a discussion with a
142 third reviewer (MM) and within the team of authors of this manuscript. Using this approach
143 each study was allocated a risk of bias rating of either low, moderate, or high.

144 *Data extraction*

145 Data were extracted directly into customized tables for characteristics of included studies
146 and results by one reviewer (MM). Information was extracted on general study
147 characteristics (lead author, year of publication, study design, country), participants
148 (number, age category (adolescents or adults), BMI, AMH, PCOS diagnostic criteria,
149 medication status), and diagnostic accuracy results (threshold, sensitivity, specificity, area
150 under the curve, and precision). Due to the timeline intensive nature of conducting
151 evidence-synthesis for an international guideline, authors were not contacted in instances
152 of missing data or for data conversions.

153 *Data synthesis*

154 Due to the heterogeneity in diagnostic criteria and/or threshold/cut off values, meta-
155 analyses (for pooled sensitivity and specificity estimates) have not been performed and thus
156 the study data are presented narratively and in tabular form. True and false positive, and
157 true and false negative, values for the diagnostic accuracy of AMH for PCOS and PCOM were
158 calculated in Review Manager 5.3 using the sensitivity and specificity data extracted from
159 included studies (MM and ECT). AMH data presented as ng/ml were converted to SI units,
160 pmol/L (conversion factor of 7.1429).

161 *Results*

162 A total of 313 potentially relevant studies were identified in the electronic database search,
163 of which 41 duplicates were excluded. The remaining 272 articles were reviewed by title and
164 abstract and 230 were excluded. Forty-two articles were retrieved for full-text screening, of
165 which 29 studies [16, 26-53] addressed diagnostic accuracy of AMH for PCOS and/or PCOM
166 and thus met the inclusion criteria for the clinical questions presented in this review, whilst

167 13 full-text articles were excluded (**Figure 1**). A table of the excluded studies with reasons
168 for their exclusion can be found in section 1.5 of the technical report for the International
169 evidence-based guideline for the assessment and management of polycystic ovary
170 syndrome [24].

171 **INSERT FIGURE 1 HERE**

172 Figure 1: PRISMA flow diagram

173

174

175 One of the 29 studies identified was a systematic review [34] and included nine of the
176 studies identified here. However, it also included studies that did not meet the inclusion
177 criteria for this evidence review, and was missing additional studies published more recently
178 that were identified by this review's search; therefore, it was not used in this systematic
179 review.

180 *Characteristics of included studies*

181 **Supplementary table 1** includes key characteristics of included studies with four addressing
182 diagnostic accuracy of AMH for PCOS and PCOM [31, 32, 38, 43], and one addressing PCOM
183 only [48]. Of the 28 studies, six studies included adolescent participants for diagnosis of
184 PCOS [32, 36, 45, 46, 48, 51] and one of these addressed PCOS and PCOM [32]. The
185 remaining 21 studies [16, 26-31, 33, 37-44, 47, 49, 50, 52, 53] included adult participants for
186 diagnosis of PCOS, where three of these addressed PCOS and PCOM [31, 38, 43]; the
187 remaining 18 studies addressed PCOS alone [16, 26-30, 33, 37, 39-42, 44, 47, 49, 50, 52, 53].
188 Of the studies in adolescents, one was in overweight and obese participants [35], and in one
189 study BMI was unclear [51]. Of the studies in adults, one included lean and obese
190 participants [27], and five studies [26, 31, 37, 52, 53] included overweight and obese
191 participants.

192 Participant numbers ranged from 31 to 633 participants for adolescents, and from 44 to 606
193 for adults. The studies were conducted across a range of settings including university
194 departments, outpatient hospital clinics and laboratories, in countries including Australia,
195 Indonesia, South Korea, Iran, Chile, USA, Turkey, Italy, Taiwan, Croatia, France, Norway, UK,
196 Germany, Denmark, China and India.

197 *Quality appraisal of included studies*

198 The six studies which included adolescent participants ranged in quality from low to high
199 risk of bias, whilst the majority of adult studies were at high risk of bias [24]. Reasons for
200 these ratings include: selection criteria were not explicitly stated; it was unclear whether
201 participants were entered into the study appropriately (randomly or consecutively); case-
202 control design; inclusion of PCOM cases among controls; and inadequacies around
203 application of index and reference tests, in particular, suboptimal choice about the best
204 compromise between sensitivity and specificity by receiver operating characteristic (ROC)
205 curve analysis. Moderate or high risk of bias was noted in interpretation of the results.

206

207 *Diagnostic accuracy of AMH for PCOS*

208

209 In adolescents, there were five studies, of which one was found to have a low risk of bias [32],
210 two were of moderate risk of bias [35, 36, 46] and two were of high risk of bias [45, 51],
211 demonstrating areas under the ROC curve of AMH for the diagnosis of PCOS, ranging from 0.5
212 to 0.88 (**Table 1**); the threshold cut-off values ranged from 25 to 44 pmol/L.

213 In adults, there were 21 studies, of which five were found to have a moderate risk of bias [29,
214 41-43, 49] and 16 were of high risk of bias [16, 26-28, 30, 31, 33, 37-40, 44, 47, 50, 52, 53],
215 demonstrating areas under the ROC curve of AMH for the diagnosis of PCOS ranging from
216 0.66 to 0.994 (**Table 1**); the threshold cut-off values ranged from 10 to 57 pmol/L. Although
217 mean serum AMH levels in adolescent and adult PCOS women were significantly higher than
218 those of non-PCOS participants in all studies, there was significant overlap between the cases
219 and controls. The sensitivity, specificity and AUC was generally higher in adults than in
220 adolescents, acknowledging that the evidence is of limited quality and that study populations

221 varied widely across studies in terms of recruitment and definitions of both PCOS and control
222 populations.

223

224 **INSERT TABLE 1 HERE**

225

226 *Diagnostic accuracy of AMH for PCOM*

227

228 In adolescents, there was one study of low risk of bias demonstrating an area under the ROC
229 of AMH for the diagnosis of PCOM of 0.87 [48] (**Table 2**); the threshold cut-off value was 50
230 pmol/L. In adults, there were four relevant studies, one of which was found to have a low risk
231 of bias [32], one of moderate risk of bias [43] and two of high risk of bias [31, 38],
232 demonstrating areas under the ROC of AMH for the diagnosis for PCOM of 0.67 to 0.92 (**Table**
233 **2**). The threshold ranged from 20 to 30 pmol/L. Although serum AMH levels in adolescent and
234 adult PCOM women are significantly higher than those of non-PCOM counterparts in all
235 studies, there is significant overlap between cases and controls.

236 **INSERT TABLE 2 HERE**

237

238

239 [Identified gaps in the AMH literature](#)

240 This systematic review presents the most up to date, rigorous synthesis of peer-reviewed
241 literature assessing whether AMH is effective for the detection of PCOM and diagnosis of
242 PCOS, in both adolescents and adults, with results informing the international guideline on
243 assessment and management of PCOS. The 28 included studies were rated with the majority
244 having a moderate, or high risk of bias. Heterogeneity was significant with identified
245 challenges including poorly defined study populations, variation across the life span, ill-
246 defined approaches to AMH cut offs and challenges with aligning with PCOM and assay
247 evolution and technical challenges.

248 The systematic review revealed significant heterogeneity in the accuracy of AMH in
249 reflecting PCOM and in assisting the diagnosis of PCOS. Key contributors to this
250 heterogeneity include the inappropriate selection of participants and the lack of well-
251 defined study populations (those with or without PCOS or features of PCOS in the control
252 populations). It is crucial that participants are entered into studies based on explicit, well
253 defined and transparent selection criteria. Study populations need to be generalisable and
254 ideally community recruited, rather than from high risk subgroups including those
255 presenting with infertility. Comparators or controls need to be very clearly and consistently
256 defined. Entrance to the studies needs to be either random or consecutive and studies need
257 to be adequately powered to detect the specified outcome. The majority of available studies
258 fail to fulfil these criteria leading to a moderate to high risk of bias and poor reliability. This
259 needs to be addressed before progress can be made in understanding the role of AMH
260 assays in PCOS.

261 Follicle development varies across the life span and is increased in adolescence, falling
262 subsequently until menopause, when oocytes are depleted. There is a need for age specific
263 cut offs for both PCOM and AMH. Here the sensitivity, specificity and area under the ROC
264 curve suggests greater accuracy of AMH in PCOS diagnosis in adults than in adolescents and
265 it may be that the role of AMH in PCOS diagnosis will align with that of PCOM. The new
266 international guidelines now recommend against the use of ultrasound in the diagnosis of
267 PCOS until 8 years post menarche (Box 1) [1-4], however more research is needed to
268 determine age specific cut offs and acceptable accuracy at given life stages. Given that AMH
269 is also not appropriate for diagnosis in adolescents or adults at present, both
270 hyperandrogenism and ovulatory dysfunction are currently required for diagnosis in
271 adolescents.

272 Another key challenge with the literature is the significant variability in the way the cut-off
273 values were defined. Traditionally in determining cut-off values in biochemical tests as
274 “normal” range, a cut-off of the 95th centile is applied to deliver 95% specificity. However, this
275 is not appropriate for defining diagnostic cut offs for a clinical condition. Here more important
276 considerations include clustering with other clinical features such as hirsutism,
277 hyperandrogenism and oligo-anovulation, or prediction of long term health outcomes such
278 as fertility. For example, establishing gestational diabetes, hypertension, or obesity cut-offs
279 were based on long-term health risks, not simply percentiles [54-56]. In the case of AMH, the
280 majority of studies defined the cut-offs at the 95th centile which is not a valid biological cut
281 off. Further research on clustering of AMH with other features of PCOS and the relationship
282 between AMH and long-term health outcomes is now vital.

283 Other considerations were the significant variability in follicle numbers and development, in
284 PCOM and in AMH across the lifespan. Levels are high in adolescence and overlap
285 considerably with those who do not have other features of PCOS. This makes it very difficult
286 to differentiate PCOS from controls on AMH levels [57]. Levels fall in later life, especially after
287 menopause [58]. Age specific reference ranges are thus vital [59] and it is likely that aligned
288 with PCOM as a diagnostic feature of PCOS, AMH will be of most use where overlap is least
289 notable, beyond the early post menarche years.

290 The relationship between AMH with PCOM was also an important consideration (Box 1).
291 Investigators have used the PCOS definition established in 2003 at the Rotterdam conference
292 [60], i.e. 12 follicles of 2-9mm diameter per ovary, to define this PCOS diagnostic criteria. This
293 cut-off suffers from the same challenges of applying the 95th centile cut offs to define PCOM
294 and is highly variable by life stage and dependent on advancing ultrasound equipment.
295 Therefore, with the latest ultrasound equipment, the new international guidelines have
296 redefined the PCOM cut offs to a threshold of ≥ 20 FNPO and have specified that ultrasound
297 defined PCOM is no longer appropriate in PCOS diagnosis within 8 years post menarche, given
298 the overlap between PCOS and controls [1-4, 12, 13]. With similar challenges in defining
299 PCOM (cut-offs at the 95th centile, changes across the life stage and technical challenges
300 mandate further research on clustering of PCOM with other features of PCOS and the
301 relationship between PCOM and long-term health outcomes.

302

303 **INSERT BOX 1 HERE**

304 In addition, there are technical issues regarding the assays for serum AMH, leading to further
305 heterogeneity in results. About one-half of the studies were performed using either the

306 Diagnostic Systems Lab (DSL) or Immunotech (IOT) assays, for which concordance in values is
307 problematic. Furthermore, these assays are not marketed anymore. There is very little data
308 with the new automated platform assays [41]. There is rising awareness on the impact of
309 sample handling, transport, and storage conditions, factors which are under-reported in the
310 literature. There is also a clear need for an international reference standard for AMH and for
311 robust independent evaluation of commercial assays in routine clinical samples with well-
312 defined sample handling and processing protocols [19]. Overall there is an urgent need for
313 international standardisation in order to improve comparability amongst assays, the
314 challenge of determining the optimal assay and the issues concerning sample storage and
315 processing need to be addressed before clinical utility can be recommended (Box 2) [1-4].

316

317 **INSERT BOX 2 HERE**

318

319

320 *Limitations*

321 A single protocol document for all 40 systematic reviews completed as part of the
322 international PCOS guideline was developed and signed off by all 70 Guideline development
323 group expert, consumer and health professional members. These protocols are publically
324 available at [https://www.monash.edu/__data/assets/pdf_file/0020/1412282/PCOS-](https://www.monash.edu/__data/assets/pdf_file/0020/1412282/PCOS-Guideline_Technical-report.pdf)
325 [Guideline_Technical-report.pdf](https://www.monash.edu/__data/assets/pdf_file/0020/1412282/PCOS-Guideline_Technical-report.pdf), however each individual protocol was not registered. This
326 review was limited to studies published in English, thus putting the review at risk of
327 language bias. Also, we did not contact study authors for missing information or data
328 conversions.

329 *Concluding remarks*

330 AMH may play a key role in the pathogenesis of PCOS, however key issues must be
331 addressed before it can be applied clinically to the detection of PCOM or in the diagnosis of
332 PCOS. These include consistently defined and appropriate study and control populations,
333 biologically relevant cut-off values that reflect clustering of clinical features and are relevant
334 to health outcomes, are life stage specific, more clearly defining PCOM on ultrasound, and
335 improved accuracy and standardisation of assays and handling procedures. With improved
336 standardisation of emerging assays and established internationally approved cut-off
337 levels/thresholds based on large scale validation in defined populations of different ages,
338 AMH may become useful in the clinical detection of PCOM and the diagnosis of PCOS.
339 However, until these issues are addressed, AMH is not clinically applicable and useful in
340 detecting PCOM or diagnosing PCOS and is not recommended outside research in the new
341 International evidence based guidelines for the assessment and management of PCOS [1-4].

342 Acknowledgements

343 We thank Estifanos Baye for performing the critical appraisals of the adolescent studies
344 included in this review.

345 Author's roles

346 M.M with input from all authors designed the search strategy, M.M ran the database
347 searches, screened articles, selected articles, performed data extraction, performed data
348 conversions, completed the statistical analyses, and contributed to the write up of the
349 manuscript. E.C.T critically appraised articles and contributed to the write up of the
350 manuscript. H.T, contributed to the write up of the manuscript. All authors assisted in
351 interpretation of the synthesised literature, critically revised the manuscript and approved
352 the final version for submission.

353 Funding

354 The guideline and associated systematic reviews were primarily funded by the Australian
355 National Health and Medical Research Council of Australia (NHMRC) supported by a
356 partnership with the European Society of Human Reproduction and Embryology and the
357 American Society for Reproductive Medicine. Guideline development group members did
358 not receive payment. Travel expenses were covered by the sponsoring organisations.

359 Disclosure statement

360 HT received NHMRC grant funding to the institution to undertake this work. RA is a
361 consultant for Medtronics, Ansh Labs, Spruce Biosciences, and Longitude Capital, and on the
362 advisory board of Martin PET Imaging. JSEL has received grants from Ferring and
363 Euroscreen. MM, ECT, DD, EHN, RJN, MA, SF, KH, SH, SO, DS, FH, SO, PD have nothing to
364 declare.

365 Highlights

- 366 • This systematic review investigates whether serum Anti-Müllerian Hormone
367 (AMH) is an effective alternative for detection of PCOM and/or diagnosis of
368 PCOS
- 369 • There is significant heterogeneity in studies conducted in adolescents and
370 adults, with a number of limitations identified
- 371 • Studies have lacked well-defined PCOS and control populations that varied
372 across the life-span; used inconsistent methods for defining cut offs, variably
373 defined PCOM in comparator studies and had methodological assay and
374 sample handling challenges

375

Outstanding Questions

- Consistently defined and appropriate study and control populations, biologically relevant cut-off values that reflect clustering of clinical features and are relevant to health outcomes, are life stage specific, more clearly defining PCOM on ultrasound, with improved accuracy and standardisation of assays and handling procedures are needed in future studies

376

377

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540 **Table 1: Diagnostic accuracy of AMH for PCOS**

Study ID	Threshold	Diagnostic criteria*	PCOS	Non-PCOS	Sensitivity	Specificity	True positive	False positive	True negative	False negative	AUC	Precision
Hart 2010	30 pmol/L	Rotterdam	64	149	53.1	69.8	34	45	104	30	0.64	CI=0.55–0.72 p=0.002
	30 pmol/L	NIH	36	177	52.8	66.1					0.61	CI=0.49–0.72 p=0.048
Kim 2016 & 2017	44.71 pmol/L	NIH	46	43	67	81					0.788	0.687-0.868 p<.0001
Sopher 2014	24.29 pmol/L	NIH	15	16	40	93.8					NR	NR
Tokmak 2015	100 pmol/L	Rotterdam Youden index	43	47	48.8	77.1					0.579	0.453-0.705 p=0.198
Yetim 2016	43.57 pmol/L	Rotterdam	53	26	81.1	92.3	43	2	24	10	0.88	CI=0.80-0.96 p<0.001
Carmina 2016	>33.57 pmol/L	Rotterdam	113	47	79	96	89	2	45	24	0.952	SD=0.014
	>33.57 pmol/L	A and B	78	47	91	96					0.982	SD=0.002
	>33.57 pmol/L	C	20	47	50	96					NR	NR
	33.57 pmol/L	D >	15	47	53	96					NR	NR
Casadei 2013	33pmol/L	NIH	22	22	95	95					0.970	CI=0.02–0.92
Cassar 2014	>30 pmol/L	Rotterdam	43	35	82	79	35	7	28	8	0.829	CI=0.736–0.923 P <0.001
Chao 2012	25pmol/L	Rotterdam	31	24	74	79	23	5	19	8	NR	NR

Dewailly 2014	28 pmol/L	Rotterdam	95	521	84.2	97.5	80	13	508	15	0.948	CI=0.915–0.982
	28 pmol/L	HA+PCOM	67	521	61.2	97.5					0.894	CI=0.852–0.936
	28 pmol/L	OA+PCOM	110	521	81.8	97.5					0.938	CI=0.908–0.969
Dewailly 2011	35 pmol/L	Rotterdam	62	66	92	97	57	2	64	5	0.973	CI=0.947–0.998
Eilertsen 2012	10 pmol/L	Rotterdam	56	206	98.2	94.8	55	11	195	1	0.992	CI=0.986-0.999
	20 pmol/L	AES	44	218	95.5	97.2					0.994	CI=0.987-1.000
Homburg 2013	48 pmol/L	Rotterdam	90	90	60	98.2	54	2	88	36	0.805	NR
Königer 2014	25 pmol/L	Rotterdam mild	21	48	71.4	89.6	15	5	43	6	0.80	CI=0.65–0.91
	25 pmol/L	Rotterdam severe	59	48	84.7	89.6	50	5	43	9	0.88	CI=0.80–0.95
Lauritsen 2014	18 pmol/L	Rotterdam	74	373	91.8	98.1	68	7	366	6	0.994	CI=0.990–0.999
Li 2010	57.14 pmol/L (8 ng/mL)	Rotterdam	47	40	61.7	70	29	12	28	18	0.664	CI=0.551–0.778
Li 2012	28 pmol/L	Rotterdam	131	61	65	62	85	23	38	46	0.68	CI=0.60–0.76 p<0.01
	30.21 pmol/L	HA+	62	61	82	64					0.82	CI=0.72–0.92 p<0.01
	26.86 pmol/L	HA-	69	61	64	62					0.66	CI=0.56–0.75 p<0.01
Pigny 2006	60 pmol/L	Rotterdam	73	96	67	92	49	8	88	24	0.851	CI=0.796–0.905
Pigny 2016	57.28 pmol/L	Rotterdam equivalent	47	48	74.5	91.7	35	4	44	12	0.944	CI=0.901–0.987
Sahmay 2013	28.14 pmol/L	Rotterdam	419	151	80	89.8	335	15	136	84	0.916	CI=0.897–0.935 p < 0.0001
Sahmay 2014	27.14 pmol/L	AES	195	411	80	80.2					0.87	0.84-0.90 p<0.001

	27.14 pmol/L	Rotterdam	228	378	81.6	85.1	186	56	322	42	0.89	0.87-0.92 p<0.001
	27.14 pmol/L	NIH	164	442	80.7	74.7					0.86	0.82-0.89 p<0.001
Saikumar 2013	23.86 pmol/L	Rotterdam	60	60	98	93	59	4	56	1	0.956	NR
Tremellen 2015	≥36 pmol/L	Rotterdam	43	113	83.7	82.3	36	20	93	7	0.917	NR
Wiweko 2014	31.79 pmol/L	Rotterdam	71	71	76.1	74.6	54	18	53	17	0.870	CI=0.81–0.92
Woo 2012	55.86 pmol/L	Rotterdam	87	53	75.9	86.8	66	7	46	21	0.868	CI=0.801-0.919
Zadehmodarres 2015	22.5 pmol/L	Rotterdam	60	57	70.37	77.36	42	13	44	18	NR	NR

541 Phenotype A, anovulation, hyperandrogenism, and PCO; Phenotype B, ANOV-PCOS, anovulatory with hyperandrogenism and normal ovaries; Phenotype C,
542 OV-PCOS, ovulatory with normal menses, hyperandrogenism, and PCO; Phenotype D, NH-PCOS, anovulatory with normal androgen levels and no symptoms
543 of hyperandrogenism and PCO; PM, PCOS mild, PCO+OA; PS, PCOS severe, all three criteria; *see table of characteristics for definition.

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552 **Table 2: Diagnostic accuracy of AMH for PCOM**

Study ID	Threshold	Diagnostic criteria	PCOS	Non-PCOS	Sensitivity	Specificity	True positive	False positive	True negative	False negative	AUC	Precision
Villarroel 2011	50.25 pmol/l	Rotterdam	25	49	84.0	83.7	21	8	41	4	0.873	CI=0.782–0.963 p<0.0001
Eilertsen 2012	20 pmol/L	Rotterdam	113	149	79.6	72.5	90	41	108	23	0.896	CI=0.855-0.937
Hart 2010	30 pmol/L	Rotterdam	75	132	54.7	72.7	41	36	96	34	0.67	CI=0.60–0.75 p<.001
Lauritsen 2014	20 pmol/L	Rotterdam	74	373	82.0	84.6	61	57	316	13	0.906	CI=0.878–0.933
Sahmay 2014	27.14 pmol/L	Unclear	Unclear	Unclear	83	87					0.92	CI=0.90–0.93 p<0.001

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Box 1. Ultrasound and PCOM recommendations- International Evidence-based Guideline [1-4]

Ultrasound should not be used for the diagnosis of PCOS in those with a gynaecological age of < 8 years (< 8 years after menarche), due to the high incidence of multi-follicular ovaries in this life stage **(CCR)**

- The threshold for PCOM should be revised regularly with advancing ultrasound technology, and age-specific cut off values for PCOM should be defined **(CCR)**
- The transvaginal ultrasound approach is preferred in the diagnosis of PCOS, if sexually active and if acceptable to the individual being assessed **(CCR)**
- Using endovaginal ultrasound transducers with a frequency bandwidth that includes 8MHz, the threshold for PCOM should be on either ovary, a follicle number per ovary of ≥ 20 and/or an ovarian volume ≥ 10 ml, ensuring no corpora lutea, cysts or dominant follicles are present **(CCR)**
- If using older technology, the threshold for PCOM could be an ovarian volume ≥ 10 ml on either ovary **(CPP)**
- In patients with irregular menstrual cycles and hyperandrogenism, an ovarian ultrasound is not necessary for PCOS diagnosis; however, ultrasound will identify the complete PCOS phenotype **(CPP)**
- In transabdominal ultrasound reporting is best focused on ovarian volume with a threshold of ≥ 10 ml, given the difficulty of reliably assessing follicle number with this approach **(CPP)**
- Clear protocols are recommended for reporting follicle number per ovary and ovarian volume on ultrasound. Recommended minimum reporting standards include:
 - Last menstrual period
 - Transducer bandwidth frequency
 - Approach/route assessed
 - Total follicle number per ovary measuring 2-9mm
 - Three dimensions and volume of each ovary
 - Reporting of endometrial thickness and appearance is preferred – 3-layer endometrial assessment may be useful to screen for endometrial pathology
 - Other ovarian and uterine pathology, as well as ovarian cysts, corpus luteum, dominant follicles \geq equal 10mm **(CPP)**
- There is a need for training in careful and meticulous follicle counting per ovary, to improve reporting **(CPP)**

555 CCR= clinical consensus recommendations; CPP= clinical practice point

Box 2. AMH recommendations- International Evidence-based Guideline [1-4].

- Serum AMH levels should not yet be used as an alternative for the detection of PCOM or as a single test for the diagnosis of PCOS **(EBR)**
- There is emerging evidence that with improved standardisation of assays and established cut off levels or thresholds based on large scale validation in populations of different ages and ethnicities, AMH assays will be more accurate in the detection of PCOM **(CPP)**

Future steps for AMH in PCOS

- PCOM needs to be consistently defined and follow international guidelines to allow comparison with AMH levels
- The inclusion of controls with PCOM should be avoided, as previously mentioned. This requires a particular statistical approach (cluster analysis).
Age-stratified thresholds need to be defined.
- Standardized optimal assays need to be applied
- AMH is a potential future substitute for detecting PCOM, however further research is needed including establishing universal threshold for elevated serum AMH level that requires validation in large populations of different ages and ethnicities.

556 EBR= evidence-based recommendation; CPP= clinical practice point

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