CHAPTER

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The Role of Antimullerian Hormone in Assisted Reproduction

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Abbreviations

ABC	ATP-binding cassette
CD	cluster of differentiation
CSC	cancer stem cell
EGF	epidermal growth factor
EMT	epithelial-to-mesenchymal transition
FTE	fallopian tube epithelium
HGSC	high-grade serous carcinoma
IGF	insulin-like growth factor
IRAK	interleukin 1 receptor-associated kinase
LH	luteinizing hormone
MET	mesenchymal-to-epithelial transition
OSE	ovarian surface epithelium
PD-L1	programed death ligand 1
TF	transcription factor
TGFB	transforming growth factor beta
TNF	tumor necrosis factor

INTRODUCTION

Since the mid-20th century, antimullerian hormone (AMH), aka Mullerian-inhibiting substance (MIS), has been known to play a vital role in fetal sexual differentiation. Alfred Jost demonstrated in 1947 in his classical castration experiments in the fetal rabbit that a testicular factor distinct from testosterone was responsible for Müllerian duct regression during male fetal sexual differentiation [1]. This putative Müllerian inhibitor was purified and shown to be produced by Sertoli cells in the testes [2,3]. In humans, by 8 weeks of gestation, AMH secreted by testicular Sertoli cells acts upon its Type II receptors located on the surface of the Müllerian ducts resulting in their apoptosis and regression. During fetal development, AMH shows a sex-dimorphic expression pattern, and in the absence of AMH the Müllerian ducts persist, giving rise to fallopian tubes, uterus, cervix, and upper part of vagina. Mutations in the AMH gene, located on chromosome 19p13.3, cause persistent Müllerian duct syndrome (PMDS), characterized by the presence of a uterus, fallopian tubes, and the upper part of the vagina in otherwise phenotypically normal males [4]. Following Alfred Jost's seminal discovery, AMH has been used clinically in the focused evaluation of intersex conditions including the diagnosis of ambiguous genitalia and cryptorchidism. For many years, the function of AMH was thought to be restricted to in utero fetal sexual differentiation until the late-20th century, when other discoveries revealed that AMH plays an important role in postnatal ovarian biology, transforming the field of reproductive medicine and assisted reproductive technology (ART) [5]. This chapter will provide an overview of AMH as an important hormone in human reproductive biology and medicine.

AMH IN OVARIAN PHYSIOLOGY

AMH is a glycoprotein that belongs to the transforming growth factor β (TGFβ) superfamily of peptide growth and differentiation factors. This family includes TGFβs, activins, inhibins, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs) [6]. While most of these ligands show a broad expression pattern and a wide range of functions, the expression of AMH appears restricted to the gonads and AMH exerts its effects only on reproductive organs [7]. It is produced in the female exclusively by granulosa cells of small and large preantral and small antral follicles [8]. Members of the TGFβ family signal through a heteromeric receptor complex, consisting of type I and type II serine/threonine kinase receptors. In general, the type I receptor is recruited into the receptor complex upon binding of the ligand to the type II receptor although BMP receptors can form ligand-independent receptor complexes [7]. AMH is secreted as a 140 kDa disulfide-linked homodimer. AMH signals through one type II receptor and three

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type I receptors. Although these AMH type I receptors (ALK2, ALK3, and ALK6) are shared by BMPs [7,9–11], signaling specificity is guaranteed by the exclusive AMHRII. Interaction of AMH with its receptors stimulates a signaling pathway which involves Smad proteins [7]. Mutations in both the AMH and AMHRII gene result in a similar phenotype in men, that is, PMDS [12]. Also, male AMHRII knockout mice have a similar phenotype to male AMH knockout (AMHKO) mice, displaying persistence of Müllerian ducts, seminiferous tubule atrophy, and Leydig cell hyperplasia [13].

The ovary begins producing AMH from about 36 weeks of gestation up until menopause [14]. The absence of AMH expression during the female fetal period is not only crucial for proper female reproductive tract development, but also for normal ovarian development. As expected, overexpression of human AMH under the control of the metallothionein promoter in transgenic mice leads to absence of a uterus and oviducts due to the action of AMH on the Müllerian ducts. However, AMH overexpression also causes oocyte loss, subsequent development of a testis-like phenotype with cord-like structures resembling seminiferous tubules, and eventually complete degeneration of the ovaries in adult transgenic females [15]. Thus, overexpression of AMH during the fetal period is detrimental for normal ovarian development. Important insights regarding the role of AMH in the adult ovary were gained from AMHKO mouse model. While lack of AMH does not affect fertility in female mice when young, increased recruitment of primordial follicles leads to their early depletion in ovaries of AMH-deficient mice [16,17]. At 4 months of age ovaries of AMHKO mice contain nearly threefold more preantral and small antral follicles and concomitantly less primordial follicles. At 13 months of age, the primordial follicle pool in AMHKO ovaries is nearly depleted and less growing follicles are present compared to wild-type mice [17]. We have since come to learn of the fundamental role AMH plays in early folliculogenesis by regulating both primordial follicular recruitment and cyclic selection of the antral follicles.

Inhibition of Primordial Follicle Recruitment

Evidence that AMH plays an important role in inhibition of primordial follicle recruitment emanates from in vitro as well as in vivo studies but appears to be species specific. In both mouse and rat neonatal ovary culture systems, ovaries cultured in the presence of AMH showed less primordial follicle recruitment compared to those cultured in the absence of AMH [18,19]. Similarly, exposure of neonatal mouse ovaries or bovine ovarian cortical strips to high levels of AMH secreted by chick gonads, by grafting the mouse ovaries underneath the chorioallantoic membrane of chick embryos, inhibited primordial follicle recruitment [20]. This effect was dependent on the presence of AMHRII, since primordial follicle recruitment was not suppressed in grafted AMHRII-deficient neonatal ovaries [20]. in vivo studies in mice lend further evidence that AMH inhibits primordial follicle recruitment. In AMHKO mice, there is increased recruitment and early depletion of primordial follicles [16,17], while AMH administration in wild-type mice leads to inhibition of follicular development as well as atresia [21].

In studies on primates and human tissue, results have been less clear cut. In primates, exposure of cultured secondary follicles of Rhesus macaques to AMH promoted while AMH antibody treatment delayed, antrum formation of growing follicles compared with controls [22], suggesting that AMH effects on primordial follicle recruitment in primates is different from rodents. In cultured human ovarian cortical strips, AMH treatment resulted in reduced primordial follicle growth in one study [23], whereas in another study AMH actually increased the number of growing primordial follicles [24]. The latter study used a higher dose of AMH, and previously cryopreserved tissue, which may explain these conflicting effects of AMH on human ovarian tissue. Moreover, AMHRII is only rarely expressed in human preantral follicles [25] while it is significantly expressed in rodent preantral follicles [26], providing further support to the notion that AMH effects on primordial follicles are species specific and may not have a significant role at this stage of follicle development in humans.

Inhibition of FSH Responsiveness

The expression pattern of AMH in the adult ovary suggests that AMH may regulate follicle-stimulating hormone (FSH)-dependent cyclic selection. AMH is produced by cumulus and mural granulosa cells of small and large preantral and small antral follicles [8]. Follicles 2–6 mm in diameter provide 60% of serum AMH. AMH expression is greatest in granulosa cells of follicles <4-mm diameter found in secondary and small antral follicles [27]. AMH is absent in larger antral stage follicles measuring >6–8 mm in diameter and thus, it is not detected in granulosa cells of preovulatory follicles. Hence, an overview of folliculogenesis might be viewed in two stages, early and late (Fig. 1). Early folliculogenesis is when in the preantral stage the follicle is functionally an AMH secreting and paracrine signaling unit. Late folliculogenesis begins after FSH receptor expression and responsiveness converts the follicle to an estradiol secreting endocrine organ using androgens as substrate. This decrease and eventual absence of AMH production in follicles destined to become larger antral follicles may

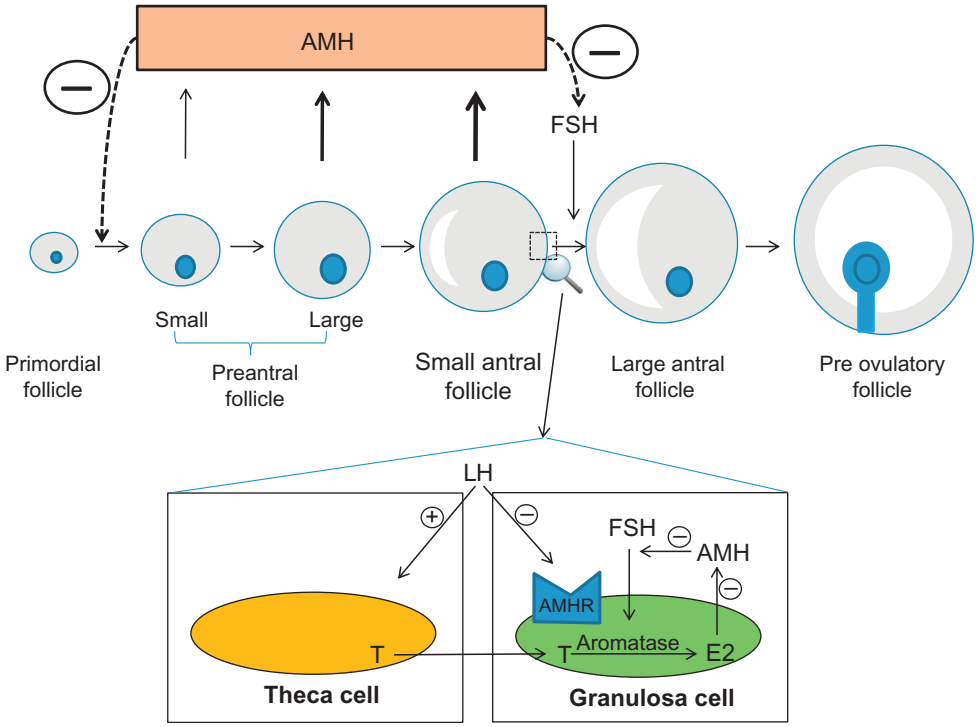

FIG. 1 AMH production and action according to follicular stage. AMH is secreted primarily from preantral and small antral follicles. The inhibitory actions of AMH are shown on the FSH-independent initial recruitment of primary follicles from the primordial follicle pool and on the FSH-dependent follicular maturation and selection of the dominant follicle by decreasing follicular sensitivity to FSH. The inset shows the interplay between AMH, FSH, and LH actions at the molecular level. AMH inhibits FSH-induced aromatase expression in granulosa cells, reducing the conversion of T to E2, which inhibits AMH in turn. In addition to stimulating theca cell to produce T, LH acts directly on granulosa cells downregulating the expression of AMHR II. *T*, testosterone; *E2*, estradiol; *LH*, luteinizing hormone; *FSH*, follicle stimulating hormone; *AMHR II*, AMH receptor II. *From Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. Reprod Biomed Online 2016;33(1):15–28.*

represent a critical mechanism by which a few individual follicles compete to become the dominant preovulatory follicle in late folliculogenesis. This is in line with previous studies showing downregulation of FSH target gene expression by AMH. Aromatase activity, encoded by the CYP19a1 gene, was decreased in ovine, rat, and rabbit fetal ovaries exposed in vitro to AMH [28,29]. Furthermore, in rat and porcine granulosa cells of growing follicles, AMH inhibited FSH-induced aromatase and LH receptor mRNA expression [30]. These results were subsequently confirmed in studies using ovine and human granulosa cells. In ovine granulosa cells isolated from small antral follicles AMH dose-dependently decreased FSH-induced estradiol production, whereas in cultured theca cells AMH inhibited LH-induced androstenedione production [31]. Indeed, in cultured human granulosa-luteal cells obtained from normo-ovulatory women undergoing IVF treatment AMH attenuated FSH-induced aromatase mRNA and protein expression, resulting in concomitant reduced estradiol production [32–34]. The inhibitory action of AMH on FSH responsiveness is corroborated by in vivo studies. In AMHKO mice, more small antral follicles are selected despite a blunted FSH surge [17,35]. Importantly, large preantral follicles, which normally are not subject to cyclic selection, were also selected in AMHKO mice at estrous, suggesting that in the absence of AMH follicles become prematurely FSH responsive [35]. The enhanced FSH sensitivity in the absence of AMH is also illustrated in a study in immature mice in which FSH levels were increased in vivo by combined GnRH antagonist and FSH treatment. Although FSH treatment also resulted in more antral follicles in wild-type mice, this effect was significantly enhanced in ovaries of treated AMHKO mice [36]. In summary, the overall effect of AMH is to reduce follicle sensitivity to FSH and this effect is not species specific.

FACTORS INFLUENCING AMH LEVELS

Various factors may affect serum AMH levels, and it is particularly important for the clinician to consider these when interpreting a patient's AMH test results to avoid inaccurate assessment of ovarian reserve [37]. The factors which may affect serum AMH levels can be divided into biological/genetic, reproductive, and environmental/lifestyle. They are summarized in Table 1. Among the reproductive factors, polycystic ovary syndrome (PCOS) is associated with elevated AMH levels [72,73], while ovarian suppression related to oral contraceptive pills or GnRH agonist administration can decrease AMH levels, with AMH levels generally returning to baseline within 3–4 months of oral contraceptive discontinuation [47,54–59]. Current pregnancy, endometriosis and history of ovarian surgery have all been associated with decreased serum AMH levels [48,49,52,53]. Several studies have shown that AMH is associated with racial/ethnic differences, with whites having higher AMH levels than Black, Chinese, and Latina [42–44].

TABLE 1 Effect of Biological, Reproductive and Environmental/Lifestyle Factors on AMH

Potential factor	Effect on AMH levels
Biological/genetic characteristics	
BRCA1 mutation carrier	Decrease [38,39]
FMR1 premutation carrier	Decrease [40,41]
Race and ethnicity	White higher than Black, Chinese, and Latina [42–44]
Systemic illness (e.g., Crohn's, SLE)	Decrease [45,46]
Reproductive factors	
Current pregnancy	Decrease [47]
Endometriosis	Decrease [48,49].
Granulosa cell tumor	Increase [50,51]
History of ovarian surgery	Decrease [52,53]
Ovarian suppression (OCPs, GnRH agonists)	Decrease [47,54–59]
Parity	Increase [43,47]
Polycystic ovarian syndrome (PCOS)	Increase [60–64]
Environmental/lifestyle	
Alcohol use	No effect [47]
Chemotherapy	Decrease [65–67]
Current smoking	Decrease [47,58,68–70]
Low vitamin D level	Decrease [71]
Past smoking	No effect [47,68]
Physical exercise	No effect [47]
Socioeconomic status	No effect [47]

Abbreviations: *FMR1*, fragile X mental retardation 1 gene; *OCPs*, oral contraceptive pills; *GnRH*, gonadotropin releasing hormone; *PCOS*, polycystic ovarian syndrome.

Moreover, genetic factors such as FMR1 premutation [40,41] and BRCA1 mutation [38,39] have been demonstrated to be associated with lower serum AMH levels. In addition, various environmental and lifestyle factors have been associated with serum AMH levels. Freeman et al. [74] were the first to report an association between AMH levels and obesity. However, while some studies reported a similar relationship, others did not find an association between obesity and AMH levels [43,47,54,58,74–79]. Current smoking has also been associated with reduced AMH levels. Sowers et al. [80] demonstrated that women who were smokers had an earlier age at menopause and a more rapid decline in AMH levels, suggesting that smoking may lead to either fewer oocytes or an earlier decline in oocyte number. In agreement with this study, Plante et al. [68] found that active smoking but not former or passive smoking was associated with decreased AMH values in late-reproductive age and perimenopausal women. The authors suggested that smoking may directly cause depletion of antral follicles but not primordial follicles, such that smoking cessation may permit repopulation of the growing follicular pool and normalization of AMH. Similar association between smoking and AMH was also recently reported by Schuh-Huerta et al. [81]. Serum AMH concentration has been demonstrated to positively correlate with serum 25-hydroxyvitamin D [25(OH)D] levels [71,82], suggesting that vitamin D deficiency is associated with lower ovarian reserve. Other environmental factors, such as alcohol consumption, socioeconomic status, and physical exercise have not been shown to be associated with AMH levels [47].

AMH UTILITY IN ART

Through its role in regulation of normal folliculogenesis AMH has emerged as a useful and well-used biomarker of reproductive aging of the ovary. The ovary begins producing AMH in utero at about 36 weeks of gestation [14], its levels rise in young women beginning in adolescence and peak at about 25 years of age, then gradually decline until reaching undetectable levels a few years before menopause. Since AMH is secreted largely by early follicles (up to 6 mm), it is relatively independent of gonadotropins which allows for testing anytime throughout the cycle [83]. While several earlier studies suggested that AMH is relatively stable throughout the menstrual cycle in normo-ovulatory women [84–88], other studies have noted significant fluctuations within one menstrual cycle [89–92]. While this issue remains highly debated, evidence suggests that these fluctuations are limited to women with high AMH levels and thus have little clinical relevance [90,93]. Clinically, AMH has evolved to become one of the most informative biochemical markers of the ovary and is considered the earliest and most sensitive marker of reproductive aging [94]. It correlates strongly with the primordial follicle pool, has an inverse correlation with chronologic age [95,96], reliably predicts ovarian response in ART [97,98] and is predictive of the timing of the onset of menopause [99–101]. The accuracy of AMH in predicting ovarian response to controlled ovarian stimulation has led to perhaps its most widely used application in ART to date: AMH-based prognostication counseling and individualization of ART stimulation protocols to optimize ovarian response. Additional areas within reproductive medicine where AMH has clinical utility include assessment of ovarian reserve in various settings (e.g., infertility evaluation, history of premature ovarian insufficiency, perimenopause, oocyte donors,

preoperative prior to ovarian surgery in reproductive age women) PCOS evaluation, fertility preservation counseling, and assessment of iatrogenic damage to egg reserve.

Age-Specific Normative AMH Values

As normal values for AMH have been demonstrated to be age-specific they are most informative in the context of chronological age. Age-specific AMH values have been provided by several studies [102–108] and are informative for the population of women presenting to fertility clinics. Thus, the reference values should be age specific and not referenced to a general population of women independent of age. As a general guideline, we consider the lower limit of age-appropriate serum AMH values for the following in 5-year age intervals to be approximately: 0.5 ng/mL for age 45, 1 ng/mL for age 40, 1.5 ng/mL for age 35, 2.5 ng/mL for age 30, and 3.0 ng/mL for age 25 [109]. These are likely to be conservative estimates as recent iterations of the most widely used AMH assay, Beckman Coulter's Gen II, report about 30%–40% higher mean values compared to these guidelines.

Prognostication and Individualization of ART

Controlled ovarian stimulation with gonadotropins for IVF/ICSI aims to obtain an adequate number of competent oocytes with the minimum risks for the woman. A large variability in ovarian response across patients given the same dose of gonadotropin is a well-recognized phenomenon. The strategy of starting all patients undergoing controlled ovarian stimulation on a standard gonadotropin dose has limitations, especially in patients at risk of poor or excessive ovarian response. For patients with a low ovarian reserve, a stimulation cycle using a standard starting dose of gonadotropin during the first 5–6 days would be expected to be associated with an elevated risk of cycle cancelation due to insufficient follicular development or no embryos/blastocysts available for transfer, and therefore would compromise success of IVF treatment. The same starting dose of gonadotropin in patients with a high ovarian reserve would be associated with markedly increased risk of early moderate/severe ovarian hyperstimulation syndrome (OHSS), a complication which can be life threatening. A strategy for individualization of gonadotropin dosing according to specific patient category characteristics could provide an optimal ovarian response, eventually leading to a reduction of safety risks and less cycle transfer cancellations, and thereby maximize the chances for successful IVF treatment outcome.

In a systematic review of studies in women undergoing controlled ovarian stimulation with gonadotropins, low AMH cut-off values (0.1–1.66 ng/mL) have been found to have sensitivities ranging between 44% and 97% and specificities ranging between 41% and 100% for prediction of poor ovarian response [8]. In a metaanalysis which included 28 studies, AMH was found to have good predictability for poor ovarian response, with an area-under the curve (AUC) of 0.78 [110]. Moreover, AMH has remarkable utility in predicting ovarian hyperstimulation in the setting of ART, with sensitivities ranging from 53% to 90.5% and specificities ranging from 70% to 94.9% when using cut-off values of 3.36–5.0 ng/mL [8].

Early follicular-phase FSH and antral follicle count (AFC), still are two parameters, have been widely used to predict ovarian reserve and response to gonadotropins. Since the study by Seifer et al. [111], in which the basal serum level of AMH was found to be associated with ovarian response to gonadotropins in women undergoing IVF treatment, many advantages of AMH over FSH (as well as over patient age, inhibin B, and estradiol) have been demonstrated [8,94,109,112,113]. Moreover, AMH offered at least similar level of accuracy and clinical value for the prediction of ovarian response to gonadotropins as AFC in three metaanalyses [110,114,115]. However, in marked contrast to these reports, four large, prospective, multicenter trials in IVF/ICSI patients consistently concluded that AMH was a better predictor of the number of oocytes retrieved as well as categorization of low and high responders than AFC [112,116–118]. Due to the limitations of AFC in terms of sonographer-dependent variability and technical aspects of ultrasound equipment [119] and the increasing advantages of AMH testing in terms of patient convenience and assay robustness, AMH is more and more being recognized as the preferred biomarker of ovarian response to controlled ovarian stimulation.

Since AMH has a good predictive ability for both poor and excessive ovarian response, prospectively designed studies have been carried out to develop AMH-based patient-tailored dosing regimens in attempt to optimize both the clinical efficacy and safety objectives for controlled ovarian stimulation. In a prospective study of 265 women, Arce et al. [120] evaluated the dose-response relationship of a novel recombinant human FSH (rhFSH; FE 999049) with respect to ovarian response in patients undergoing controlled ovarian stimulation for IVF/ICSI and studied the influence of initial AMH concentrations on the dose-response curve. As expected, the number of oocytes retrieved increased in an rhFSH dose-dependent manner. Importantly, they found that the slopes of the rhFSH dose-response curves differed significantly between the two AMH strata, demonstrating that a 10% increase in dose resulted in 0.5 (95% confidence interval 0.2–0.7) and 1.0 (95% confidence interval 0.7–1.3) more oocytes in the low and high AMH stratum, respectively [120]. A recent randomized, multicenter

noninferiority trial which included 1329 women compared the efficacy and safety of follitropin delta, a new human recombinant FSH, with individualized dosing based on serum AMH and body weight, with conventional follitropin alfa dosing for ovarian stimulation in women undergoing IVF [121]. Without any dose adjustments during stimulation, individualized follitropin delta dosing resulted in more women within the prespecified targeted ovarian response of 8–14 retrieved oocytes, with fewer clinically relevant cases of both poor and excessive ovarian responses, and a reduced need for OHSS preventive measures. This proof-of-concept study lends support to individualized dosing strategy according to pretreatment AMH as means of improved safety while maintaining efficacy compared with conventional ovarian stimulation.

Despite its strong correlation with ovarian response to stimulation in ART, AMH is a poor predictor of nonpregnancy with sensitivities between 19% and 66%, and specificities between 55% and 89% when using cut-offs ranging from <0.1 to 1.66 ng/mL [115]. Notably, a recent study of the Society of Assisted Reproductive Technology (SART) database found that while women with ultralow AMH (<0.16 ng/mL) had 54% cycle cancelation rate, the overall live birth rate per cycle start was 9.5% [122]. Similarly, AMH is a poor predictor of pregnancy and live birth following ART, with two recent metaanalyses showing the AUC for AMH as predictor of clinical pregnancy and live birth to be 0.63 and 0.61, respectively [123,124]. The sensitivities and specificities for AMH in predicting clinical pregnancy ranged from 34.4% to 86.2% and 26% to 78.5%, respectively, with cut-offs ranging between 1.0 and 3.22 ng/mL [124]. Although tempting to provide a threshold for access to clinical programs thereby ensuring only good prognosis patients are treated, it is important to remember that a large number of couples who could be successfully treated would be excluded from IVF/ICSI even if a low AMH threshold was used [125]. Moreover, AMH values were not associated with fecundability in unassisted conceptions in a cohort of fecund women with a history of one or two prior losses [126]. These data are consistent with AMH being a predictive marker of oocyte quantity but not quality.

Polycystic Ovarian Syndrome

Polycystic ovarian syndrome is the most common endocrine reproductive disorder, affecting 5%–8% of women. It has been suggested that AMH plays an important role in the pathogenesis of PCOS [127]. It is well known that PCOS ovaries comprise a higher number of preantral and small antral follicles [128–130], indicating arrest of follicular development at the stage when AMH production is greatest. Several studies have shown that serum AMH levels are elevated in PCOS women compared with women with normal ovaries [131–134]. In addition, concentration of AMH in follicular fluid from anovulatory PCOS women was found to be fivefold greater compared with ovulatory women [135]. Moreover, granulosa cells of polycystic ovaries have increased AMH mRNA expression [136], and AMH production per granulosa cell is increased on average 75-fold in granulosa cells of anovulatory PCOS compared with normal ovaries [131]. These data suggest that it is not only the increased number of follicles, with resultant increased granulosa cell mass, but also greater production by individual granulosa cells that is underlying AMH overproduction in PCOS. Since AMH levels are abnormally elevated in PCOS, AMH has been suggested as a useful marker of the disease. A cut-off of AMH >5.0 ng/mL has been shown to be highly diagnostic of PCOS (AUC 0.973, sensitivity 92%, specificity 97%) and suggested to be incorporated as a diagnostic criterion for this syndrome [72,73]. Moreover, it appears that AMH is not merely a biomarker which is elevated in PCOS but also plays a role in its pathogenesis. Substantial evidence indicates that AMH correlates strongly with the severity of various manifestations of PCOS, including polycystic ovarian morphology (PCOM), hyperandrogenism, and oligo/anovulation [60–64,137]. Moreover, elevated serum AMH concentrations are predictive of poor response to various treatments of PCOS including weight loss, ovulation induction, and laparoscopic ovarian drilling, while improvement in various clinical parameters following treatment is associated with serum AMH decline, further supporting an important role for AMH in the pathophysiology of this syndrome [127]. Thus, AMH testing should be considered in the routine workup of PCOS patients and could aid in establishing the diagnosis, provide insight to the severity of the syndrome and potential treatment resistance, as well as alerting physicians to the increased risk of OHSS if ovulation induction is being considered.

Fertility Preservation

As many young girls and women get successfully cured and survive into adulthood by life-saving, but often gonadotoxic treatments, subsequent reproductive health has become a major quality of life issue. Consequently, predicting which patients may lose their fertility and/or ovarian function as a result of treatment has become of increasing importance. Chemotherapy and radiotherapy can both have detrimental effects on ovarian function and consequently AMH levels, with the extent of injury dependent on patient-related factors, such as patient age and actual ovarian reserve at the

beginning of treatment, and treatment-related factors, such as treatment gonadotoxicity (i.e., type of cytotoxic drugs and cumulative dose) [138]. Pre- and posttreatment AMH testing in young cancer patients has become a useful tool for assessment of iatrogenic damage to the ovarian follicular reserve inflicted by gonadotoxic chemotherapy agents or pelvic irradiation and is increasingly utilized in fertility preservation counseling and strategies. Studies have demonstrated that women with higher pretreatment AMH levels have higher postchemotherapy AMH levels and display faster recovery rate in AMH levels once treatment had been completed. [65,66]. Importantly, two studies involving breast cancer patients have provided prognostic tools which allow clinicians to inform patients more precisely regarding expected postchemotherapy ovarian function using pretreatment AMH levels, taking into consideration other important variables [67,139]. The first prognostic tool demonstrated that all women, regardless of age, with AMH levels of $<0.54\,ng/mL$ will have amenorrhea posttreatment [67]. The second tool is a prognostic scoring system based on a patient's age, pretreatment AMH levels and BMI with 1 point awarded for each of age <40 years, AMH level $>0.7\,ng/mL$ and BMI $>25\,kg/m^2$ [139]. The time to recovery from completion of chemotherapy and likelihood of recovery of ovarian function improves with increasing number of points. For example, menses returned in 75% of women with 3 points by 160 days whereas only 25% of women with 0 points had a return of ovarian function by 221 days [139]. Since both of these prognostic systems were developed in breast cancer patients, and the systemic effects of different types of cancer and their specific treatment regimens vary, one should not generalize the results above to all cancers. Therefore, further research into other types of malignancies and chemotherapy regimens is required to construct a panel of individualized prognostic tools which would provide personalized information on the risk of gonad toxicity and on the requirement of fertility-preservation procedures. Moreover, since the primary outcome of the research thus far has been posttreatment amenorrhea, more research studies should be focused on the ability of pretreatment AMH levels to predict subsequent fertility. Information on this important outcome would be paramount in guiding fertility preservation counseling and treatment.

THE AMH ASSAY: POTENTIAL PITFALLS AND SOLUTIONS

The main limitations of the AMH test relate to variability between AMH assays and lack of standardized international assay. Prior to 2010, two different assays developed independently by Diagnostic Systems Laboratory (DSL) and Immunotech (IOT) were used. These assays had different antibodies and reported very different results, using different units. The IOT assay produced AMH concentrations about 40% higher compared with DSL, rendering comparison between studies difficult [140]. That problem was thought to be resolved by the manufacture of both ELISAs by the same company (Beckman Coulter) and the development of a new assay (GenII) that combines the best features of both [141]. However, several studies have demonstrated intra/interassay differences, between laboratory differences and sample stability and storage issues related to the GenII assay [142]. When 10 laboratories tested 20 serum samples with the same GenII assay, the reproducibility within each lab was good but wide variability in average value results relative to the consensus was observed between different laboratories [143]. It was realized that the GenII assay suffered from interference with AMH binding by complement when fresh and undiluted samples were assayed, affecting result interpretation [144]. The introduction of two widely available automated AMH assay platforms (Roche Elecsys and Beckman Coulter Access2) using the same antibody combination appear to be resolved the main issues concerning widespread clinical testing, even though there is currently no international standard for AMH and that the two assays are marketed by different corporations, using different technologies, and different sources of standardization [145]. These principal automated assays offer improvement of greater precision (fourfold), faster turnaround time (18 min vs. 6 h), and greater sensitivity (10-fold) compared to current ELISA-based assays [146,147]. These new platforms are being actively used outside the United States in Europe and Asia. Most recently, one such automated platform received FDA clearance for determining ovarian reserve (http://www.fdanews.com/articles/179932-fda-grants-clearance-to-roches-anti-mullerian-hormone-test-elecsys). In the meantime, it is important to be cautious in combining AMH data from different trials for analysis as well as in translating AMH cut-off levels from studies into clinical practice.

SUMMARY

The last 15 years have witnessed AMH emerge from the bench to the bedside, becoming one of the most informative biochemical markers of the ovary with clinical utility and therapeutic application. It is considered the earliest and most sensitive marker of reproductive aging, it correlates strongly with the primordial follicle pool, has an inverse correlation with chronologic age, reliably predicts ovarian response in ART and is predictive of the timing of the onset of menopause. The accuracy of AMH in predicting ovarian response to controlled ovarian stimulation has led to perhaps its most widely used

clinical application to date: AMH-based prognostication counseling and individualization of ART stimulation protocols to optimize ovarian response and minimize associated hyperstimulation risks. In addition to ART, AMH has clinical utility in PCOS evaluation and treatment, fertility preservation counseling, and assessment of ovarian reserve in several other settings. A better understanding of AMH pathophysiology may lead to the development of new pharmacological treatments using AMH agonists and/or antagonists for a host of reproductive challenges. These include the possible retardation of ovarian aging, menopause, as a chemoprotectant for fertility preservation prior to chemotherapy, as a tool to better control ovarian response to ART and/or to mitigate the deleterious effects of PCOS.

Glossary

Cancer stem cells → Cancer cells possessing characteristics of stem cells.
Cell plasticity → The ability to reversibly assume different cellular phenotypes.
Chemoresistance → A characteristic of cancer cells describing their failure to respond to the toxic effects of chemotherapeutic drugs.
Epithelial-to-mesenchymal transition → The process of an epithelial cell acquiring the phenotype of a mesenchymal cell.
Follicular fluid → The fluid filling the antrum of the ovarian follicle.
Mesenchymal-to-epithelial transition → The process of a mesenchymal cell acquiring the phenotype of an epithelial cell.
Metastasis → The process of cancer cells in a primary tumor disseminating to other tissues or organs, resulting in growth of secondary tumors.
Re-epithelialization → Re-establishing the epithelial barrier.
Stemness → Characteristic(s) associated with stem cells.

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