REVIEW

Potential therapeutic applications of human anti-Müllerian hormone (AMH) analogues in reproductive medicine

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Abstract Members of the transforming growth factor-beta (TGF-beta) superfamily are key regulators of various physiological processes. Anti-Müllerian hormone (AMH) which is also commonly known as Müllerian-inhibiting substance (MIS) is a member of the TGF-beta superfamily and an important regulator of reproductive organ differentiation and ovarian follicular development. While AMH has been used for diagnostic purposes as a biomarker for over 15 years, new potential therapeutic applications of recombinant human AMH analogues are now emerging as pharmacologic agents in reproductive medicine. Therapeutic uses of AMH in gonadal tissue may provide a unique opportunity to address a broad range of reproductive themes, like contraception, ovulation induction, onset of menopause, and fertility preservation, as well as specific disease conditions, such as polycystic ovarian syndrome (PCOS) and cancers of the

reproductive tract. This review explores the most promising therapeutic applications for a novel class of drugs known as AMH analogues with agonist and antagonist functions.

Keywords Anti-Müllerian hormone · Müllerian inhibiting substance · Fertility treatment · Contraception · Ovulation induction · Menopause · Fertility preservation · Polycystic ovarian syndrome · Ovarian cancer · Endometrial cancer · AMH agonist · AMH antagonist · AMH analogues

Background

Biology of AMH

The TGF beta family includes TGF betas, AMH, activins, inhibins, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs). Most of these ligands demonstrate a wide expression pattern and broad range of functions. The functions of AMH, in contrast, appear specifically directed toward the reproductive system, regulating and refining reproductive function [1].

AMH was initially discovered because of its role in Müllerian duct regression during male fetal development. In males, AMH is prenatally and postnatally produced by Sertoli cells. Its serum levels remain elevated until puberty, and then rapidly decline during the transition to adulthood. In testes, AMH regulates Leydig cell androgen steroidogenesis by inhibiting transcription of cytochrome P450 17 α -hydroxylase/C17–20 lyase and aromatase [2]. Persistent Müllerian duct syndrome (PMDS) is an autosomal recessive disorder of male sexual development, caused by mutations in the AMH gene on chromosome 19p13 or the AMH type II

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receptor (AMHR2) gene on chromosome 12q13 [3]. Affected males have normal male reproductive organs, often are affected by cryptorchidism, and exhibit uteri and fallopian tubes.

In females, AMH is produced postnatally by granulosa cells; levels gradually increase, with peak levels coinciding with peak fertility in the mid-20s, and decline thereafter becoming undetectable at time of functional menopause. In ovaries, AMH inhibits primordial follicle recruitment [4], meiosis II [5], granulosa cell division, and progesterone production [6].

The AMH ligand-receptor system acts via AMHR2 in mural and cumulus granulosa cells of small and large pre-antral follicles and small antral follicles, and as a leading negative paracrine growth factor, playing a fundamental role in early and late folliculogenesis by regulating primordial follicle recruitment and FSH-dependent cyclic selection of antral follicles. AMH, essentially, functions as a gatekeeper for the rate of depletion of primordial follicles and selection of maturing follicles, utilizing a BMP-like signaling pathway through AMHR2 and type I receptors, activating Smad 1/5/8, which translocates to the nucleus to regulate gene expression within granulosa cells. AMH concentration in follicular fluid is inversely correlated with granulosa cell proliferation, although normal physiology is disrupted with advancing age and in PCOS [7, 8].

Beyond gonads, AMH and its receptor are found in the prostate [9], in ductal epithelium of the mammary gland [10], and in endometrium [11]. Additionally, AMH appears to act directly on GnRH neurons, suggesting regulatory functions at multiple sites along the hypothalamic-pituitary-gonadal axis [12]. Finally, AMHR2 is highly expressed in the adrenal gland and to a lesser degree in the pancreas and spleen, though its function in these tissues has not been investigated [13].

Current diagnostic clinical applications of AMH

Following its initial discovery in human ovarian follicular fluid [14] and realization of its clinical utility as an early ovarian reserve marker in 2002 [15], AMH's applications as a diagnostic biomarker in clinical medicine have expanded [1]. Currently, AMH is clinically utilized in neonatology and pediatrics as a marker of fetal sexual differentiation in cases of ambiguous genitalia, cryptorchidism, and pubertal delay. In reproductive endocrinology, AMH is utilized to measure functional ovarian reserve and gauge reproductive aging, to individualize controlled ovarian hyperstimulation protocols, to guide fertility preservation, and to aid in the diagnosis of PCOS, of diminished ovarian reserve (DOR) and of hypogonadism in both men and women [1, 16–24]. Finally, in oncology AMH levels are measured to diagnose and monitor recurrence of granulosa cell and sex cord tumors [25, 26]. Recent studies have also identified AMH as a potential biomarker for breast cancer risk stratification in pre-menopausal women [27–29].

While particularly for treatment of various cancers the potential of AMH analogues as therapeutic agents has long been recognized [30–32], no AMH analogue has ever been brought to clinical trials. With increasing understanding that AMH analogues may in several ways also regulate fertility, renewed interest has become apparent in potentially bringing them to clinical trials. This review explores some of the most promising potential therapeutic applications for AMH analogues (Table 1).

Potential therapeutic clinical applications of AMH analogues

Fertility-related applications

Contraceptive/regulation of follicular recruitment and delay of menopause

Animal studies in AMH-deficient mice suggested the hormone's role in regulation of primordial follicle recruitment [33]. Follow-up investigations in a neonatal murine ovary culture system confirmed that AMH acts as potent inhibitor of primordial follicle recruitment [4]. In vitro experiments in human ovarian tissue subsequently demonstrated that administration of recombinant AMH suppressed the initiation of primordial follicle recruitment [34].

These observations led us and others to hypothesize that, as a mode of reversible contraception, exogenous administration of AMH could be used to temporarily halt primordial follicular recruitment, and to potentially delay onset of menopause. We then demonstrated in an in vivo murine model that AMH administration decreased follicular development and atresia, inhibited ovulation, and, thereby, interrupted the estrous cycle [35]. Treated for 4 weeks with AMH, both pre-pubertal and reproductive age female mice showed increased proportions of primordial and primary follicles, decreased proportions of antral and atretic follicles, and fewer corpora lutea in comparison to vehicle-treated mice. In addition to inhibiting initial follicular recruitment from the primordial pool, AMH diminished follicular atresia, with lower rates of DNA fragmentation demonstrated by TUNEL assay. AMH treatment also disrupted estrous cyclicity, decreased estradiol and elevated LH levels, and decreased the expression of steroidogenic acute regulatory protein, luteinizing hormone receptor, and aromatase messenger RNA (mRNA) levels, while increasing androgen receptor expression. Above actions were shown to be mediated through induction of two miRNAs, miR-181a and miR-181b, which in turn regulate FSH signaling during folliculogenesis.

Other investigators confirmed and extended this hypothesis by demonstrating that with exogenous administration of AMH, effective contraception could be achieved, while retaining normal ovarian reserve [36]. AMH-treated mice demonstrated small ovaries without growing follicles but a

normal pool of primordial follicles, disrupted estrous cycling, and hypergonadotropic hypogonadism. They also did not produce litters when paired with male breeders. Importantly, upon discontinuation of AMH administration, folliculogenesis resumed normally, suggesting reversibility of AMH's contraceptive effects [36].

Mathematical models of exogenous AMH administration for contraceptive use or to delay the onset of menopause have been published [37]. These models suggest that administration of sufficient AMH during peak female reproductive years may sufficiently reduce recruitment of follicles from the primordial pool to offer effective contraception and prolongation of the reproductive lifespan for approximately the same duration as AMH has been administered.

This concept is also supported by the clinical observation that women with PCOS, who are characterized by relatively high AMH values, even at advanced ages often demonstrate relatively high functional ovarian reserve and prolonged reproductive lifespan—on average 2 years beyond normo-ovulatory women [38, 39].

Ovarian protection from gonadotoxins

Via negative feedback loops, AMH and other suppressive factors produced by growing follicles maintain most primordial follicles in a dormant state, while allowing activation of only small cohorts of follicles. This equilibrium is disrupted by administration of chemotherapeutic agents, such as cyclophosphamide, which have been shown to exert gonadotoxic effects by inducing apoptosis in growing follicles and upregulating the PI3K/PTEN/Akt pathway. Activation of this pathway reduces inhibition of primordial follicle recruitment. Subsequent rapid recruitment and apoptosis of growing follicles then leads to rapid “burnout” of the ovarian reserve, mostly made up of primordial follicles [40].

When co-administered with gonadotoxic chemotherapy, investigational drugs, such as AS101, mTOR inhibitors like INK128, and everolimus (RAD001), which downregulate the PI3K/PTEN/Akt pathway, preserve ovarian reserve by maintaining primordial follicles in a dormant state [40, 41]. Similarly, AMH co-administration together with chemotherapeutic drugs was recently shown to protect the ovarian reserve by halting recruitment of primordial follicles [36]. These studies provide proof of concept in animal models that protection of the ovarian reserve from gonadotoxins is feasible, and strongly suggest follow-up studies in non-human primate or even human clinical trials.

Improving oocyte yield and follicle synchronization for IVF

Women with low functional ovarian reserve achieve significantly lower live birth rates following in vitro fertilization (IVF) than women with all other infertility diagnoses [42].

Beyond female age, the second most important predictor of live birth is the number of retrieved oocytes, which in turn determines the availability of transferrable embryos [43–45].

Diminished ovarian reserve is characterized by low oocyte yields following even maximum dosages of gonadotropins and by asynchronous follicle development. Asynchronous folliculogenesis poses numerous challenges in IVF, including either premature or delayed follicle maturation and luteinization. Current strategies used to improve follicle synchrony involve steroid hormone pre-treatments prior to gonadotropin stimulation for IVF but yielded mixed results [46]. Recently described strategies to increase oocyte yield [47] and to avoid premature luteinization [48] highlight the need and opportunity to improve IVF outcomes among infertile women with low functional ovarian reserve.

While most established infertility treatments pharmacologically target the gonadotropin-sensitive last 14-days of follicle maturation, future therapeutic interventions will have to be directed at earlier stages of follicle maturation if further improvements in IVF live birth rates are to be realized [49, 50]. Administration of AMH has the potential to improve oocyte yield and synchronization of follicle development when administered prior to controlled ovarian hyperstimulation with gonadotropins. This concept was recently confirmed experimentally: Mice pre-treated with AMH prior to superovulation produced more oocytes and demonstrated increased antral follicle counts [35]. A recent clinical study also suggested positive AMH effects on pregnancy and live birth chances following IVF. Interestingly, these effects were not linear since best outcomes were achieved even at very advanced ages at intermediate AMH levels [44].

Endometriosis

Endometriosis is a benign chronic disease characterized by endometrial tissue growing outside of its physiological location in the uterus, often causing pain, adhesions, and subfertility. The human endometrium has been shown to have a functional AMH signal transduction system. Endometrium and endometriotic lesion tissues obtained from women with endometriosis demonstrate elevated mRNA levels and protein expression of both AMH as well as AMHR2 [51]. Exogenous administration of AMH appears to decrease viability of cultured human endometrial stromal cells and increase their apoptosis [11]. Similarly, in vitro experiments in human ectopic endometrial cell lines indicate that AMH inhibits cell growth and induces autophagy, cell cycle arrest, and apoptosis [52–54]. Combined, these studies indicate that AMH agonists may have a potential therapeutic role in endometriosis patients.

Treatment of PCOS w/AMH antagonists

High AMH levels are a typical finding in PCOS [8, 21, 55–60]. While not currently part of the Rotterdam diagnostic

criteria of PCOS, AMH levels are increasingly viewed as an objective biomarker for the syndrome [61]. Elevated AMH levels appear to be fundamental to the suppression of FSH-dependent maturation of antral follicles, thus preventing emergence of a dominant follicle. Traditional approaches to ovulation induction in PCOS have relied on overcoming this suppression by either destroying the pre-antral and antral follicle pool, thereby lowering AMH levels (i.e., ovarian wedge resection or diathermy), or raising serum FSH levels (i.e., directly, by administering gonadotropins, or indirectly, with selective estrogen receptor modulators and aromatase inhibitors).

Both approaches may cause side effects and complications. Destroying the pre-antral and antral follicle pool requires surgery, while superovulation carries the risk of multiple gestations and ovarian hyperstimulation syndrome. Another medical approach applied in insulin-resistant women has utilized metformin and has been associated with increased ovulation with the lowering of AMH serum levels. AMH antagonists could, therefore, offer an excellent therapeutic option for ovulation induction in PCOS that potentially avoids these complications by enabling selection of a single dominant follicle (Fig. 1). This hypothesis, of course, should be tested in appropriately designed clinical trials which establish effective dosing regimens to achieve mono-ovulation.

As it appears to increase GnRH neuron-dependent LH pulsatility and secretion, AMH has recently also been implicated in the neuroendocrine pathophysiology of PCOS [12]. These findings suggest that modulating AMH-dependent regulation of GnRH release may offer an additional therapeutic option in PCOS.

Finally, the high level of AMHR2 expression in the adrenal gland [13] suggests that AMH may play a still unknown role in adrenal hyper-androgenism, frequently seen in PCOS, and in adrenal hypo-androgenism, recently described in a new hypo-androgenic PCOS phenotype [60, 64] and a frequently observed finding in women with low functional ovarian reserve [65]. Our group recently in a number of publications pointed out the hormonal interplay of adrenals and ovaries in the pathogenesis of PCOS and low functional ovarian reserve [60, 64–68]. The surprisingly dense presence of AMH receptors in adrenals raises the intriguing possibility that AMH may play a yet unrecognized, though significant, role in the hormonal interplay between adrenals and ovaries. Since adrenals and ovaries share a common embryonic primordium [69], this would not be too much of a surprise.

Clinical applications in the embryology laboratory

As additions to tissue culture and in vitro maturation culture media in the embryology laboratory, AMH analogues may also have a role in activation and suppression of follicle maturation in vitro. The goal of fertility preservation

in young women via ovarian tissue cryopreservation prior to cancer treatment has been to maintain follicular dormancy to preserve the follicle reserve in ovarian cortical grafts. Indeed, AMH supplementation during ovary tissue cryopreservation/thawing has been demonstrated to reduce follicle apoptosis in a mouse model [70]. Conversely, strategies which stimulate activation of dormant follicles in cortical tissue prior to transplantation have recently been implemented with the goal of maximizing shortly after autotransplantation of the graft yields of mature oocytes and, therefore, pregnancy chances [71].

Sporadic success was recently also reported with in vitro activation of ovarian cortical tissue from young women with primary ovarian insufficiency and from women who underwent fertility preservation prior to cancer treatment [72, 73]. Disrupting Hippo signaling by fragmentation of ovarian tissue, followed by stimulation of Akt signaling with PTEN inhibitors and PI3K activators, a group of Japanese and US investigators in vitro activated dormant follicles in surgically removed ovarian tissues of women with primary ovarian insufficiency. Subsequent autotransplantation and IVF treatment resulted in a live birth [72]. A second group of investigators extended use of PTEN inhibitors to human ovarian cortical tissue of cancer patients who underwent fertility preservation to increase the pool of viable activated primordial follicles [73]. These strategies involving activation with PTEN inhibitors still face obstacles including concerns about efficiency as well as follicle health.

Development of a human system which enables in vitro growth and maturation of fertilizable oocytes from primordial follicles appears close, and has already been realized in the mouse [74, 75]. Availability of AMH analogues is likely to facilitate further development of such systems, and their clinical adoption.

Oncologic applications

Pharmaceutical and biotechnology companies have prioritized targeting the TGF beta signaling pathway for treatment of multiple human cancers, fibrosis, and inflammatory disorders [76, 77]. Because overexpression of TGF betas in these diseases contributes to abnormal cell growth, various TGF beta inhibitors including small-molecule inhibitors, monoclonal antibodies, and gene expression inhibitors have reached clinical trials [77]. As most tumors of the reproductive tract originate from Müllerian duct-derived tissues, and AMH is known to cause regression of the Müllerian duct in male embryos, it is understandable that AMH would inhibit the growth of reproductive tract tumors. Since, to inhibit cell division and induce apoptosis, AMH acts via tissue-specific receptors, it has emerged as a promising agent for treatment of multiple reproductive tract cancers [31, 32]. Additionally, other

Fig. 1 Potential drug targets to control follicle development. **a** Inhibition of initial follicle recruitment by AMH agonists (applications include contraception, delay of onset of menopause, fertility preservation, and

infertility treatment). **b** Releasing arrested selection of a dominant follicle by AMH antagonists (applications include ovulation induction in PCOS). Modified from [50, 62, 63]

chemotherapeutic agents, immunotherapy, and tumor-directed imaging may be delivered in a tissue-specific manner by targeting AMHR2, which is highly expressed by a variety of reproductive tract cancers and much less so in other tissues [78, 79].

Ovarian cancer

AMH was first proposed as a potential therapy for ovarian cancer based on similar histological appearance of ovarian adenocarcinomas arising from coelomic epithelium and embryonic Müllerian ducts [80]. In experiments in rodent models, AMH has been shown to inhibit epithelial ovarian cancer cell proliferation in vitro and in vivo (previously reviewed in [31, 32, 81]).

Experiments in cell lines and in rodents have shown growth inhibition of xenotransplanted human ovarian cancer cells, mouse ovarian carcinoma (MOVCA) cells [82], and tumor-initiating stem-like cells. Perhaps the most convincing data, however, comes from human ovarian cancer cell lines and ascite cells from patients with advanced-stage ovarian papillary serous cystadenocarcinoma [83]. This study found that most patients' cells expressed AMHR2, and their growth was in vitro inhibited by recombinant AMH.

Endometrial and cervical cancer

The uterine endometrium arises from coelomic epithelium and expresses AMHR2, as do human endometrial cancer cell lines. AMH inhibits growth of AN3CA and KLE endometrial cancer cell lines, which express AMHR2 via an increase in cell cycle arrest and apoptosis [32, 84].

The cervix also arises from coelomic epithelium. Normal cervical tissue, cervical cancer tissue, and cancer cell lines express AMHR2 [85, 86]. AMH exposure leads in vitro to receptor-mediated growth inhibition of human papilloma virus-transformed and non-human papilloma virus-transformed cervical cancer cell lines cells [85, 86].

Table 1 Potential therapeutic roles for AMH analogues

- Retarding ovarian aging
- Delaying the onset of menopause
- Reversible contraception
- Ovarian chemoprotection for fertility preservation from cancer therapies
- Treatment of PCOS
- Treatment of endometriosis and adenomyosis
- Treatment of ovarian and endometrial cancer

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Additionally, suppression of C33A cervical cancer cell line growth appears to be related to induction of p16, p130, and p107 tumor suppressor proteins by AMH [86].

Breast cancer

AMHR2 expression has been documented in both normal human breast tissue and in various pathologic conditions of the breast including cancer cell lines, fibroadenoma, and ductal adenocarcinomas [87]. Breast cancer cell growth is inhibited by AMH in vitro and in vivo in mice by interfering with cell cycle progression and induction of apoptosis [10, 87]. These actions appear to be mediated by activation of the nuclear factor-kappa B pathway which leads to expression of B-cell translocation gene 2, IFN regulatory factor-1, and chemokine Gro-beta as well as by independent activation of the Smad1 pathway [88]. Paradoxically, pre-menopausal women with elevated serum AMH levels appear to be at increased risk for breast cancer [27–29]. Therefore, further research is needed to parse the relationships between excessive AMH production, a typical finding in PCOS, other PCOS related co-morbidities, and breast cancer risk.

Prostate cancer

AMHR2 is expressed in the prostate gland, prostate cancer cell lines, and human prostate adenocarcinomas [9]. AMH has been shown to inhibit the growth of human prostate cancer cell lines [89]. Additionally, because AMH suppresses testicular testosterone production and regulates androgen-induced gene expression and growth in prostate cancer cells, it may act via several synergistic mechanisms in the treatment of prostate cancer [90].

Ocular melanoma

Beyond the reproductive tract, AMH has been shown in nude mice to inhibit growth of human ocular melanoma cell lines both in vitro and in vivo [91].

Summary

In this review, we have presented the conceptual foundation for a novel class of drugs with several meaningful potential clinical applications in reproductive medicine. These include novel and unique therapeutic approaches to reversible non-steroidal contraception, delaying menopause, chemoprotection for fertility preservation in women planning to undergo adjunctive treatment for cancer, and treatment of PCOS and reproductive system cancers.

Drugs which modulate TGF-beta pathways, specifically AMH analogues, have the potential to transform reproductive

medicine as much as the introduction of hormonal contraceptives, gonadotropins, GnRH analogues, and aromatase inhibitors did. For treatment of a spectrum of reproductive disorders, their anticipated effects are, likely, robust, yet more localized and targeted in mechanism of action and, therefore, likely to pose fewer side effects than existing therapies.

While recombinant human AMH has been synthesized in Chinese hamster ovary cells for over two decades [92–94], newer manufacturing techniques may offer technical advantages in recombinant hormone production [95, 96]. Therefore, the time for the pharmaceutical industry to manufacture clinical grade AMH analogues for clinical trials is now.

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Compliance with ethical standards

Competing interests The authors declare pending and already awarded US patents involving diagnostic and therapeutic applications of AMH. D.B.S. received royalties from a licensing agreement between Rutgers Medical School/MGH and Beckman Coulter for the use of AMH in determining ovarian reserve.

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