

Anti-Müllerian Hormone in Fertility Preservation: Clinical and Therapeutic Applications

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ABSTRACT: Anti-Müllerian hormone (AMH) is a member of the transforming growth factor (TGF)-beta family and a key regulator of sexual differentiation and folliculogenesis. While the serum AMH level has been used in reproductive medicine as a biomarker of quantitative ovarian reserve for more than 20 years, new potential therapeutic applications of recombinant AMH are emerging, notably in the field of oncofertility. Indeed, it is well known that chemotherapy, used to treat cancer, induces ovarian follicular depletion and subsequent infertility. Animal models have been used widely to understand the effects of different cytotoxic agents on ovarian function, and several hypotheses regarding chemotherapy gonadotoxicity have been proposed, that is, it might have a direct detrimental effect on the primordial follicles constituting the ovarian reserve and/or on the pool of growing follicles secreting AMH. Recently, a new mechanism of chemotherapy-induced follicular depletion, called the “burn-out effect,” has been proposed. According to this theory, chemotherapeutic agents may lead to a massive growth of dormant follicles which are then destroyed. As AMH is one of the factors regulating the recruitment of primordial follicles from the ovarian reserve, recombinant AMH administration concomitant with chemotherapy might limit follicular depletion, therefore representing a promising option for preserving fertility in women suffering from cancer. This review reports on the potential usefulness of AMH measurement as well as AMH’s role as a therapeutic agent in the field of female fertility preservation.

KEYWORDS: Fertility preservation, AMH

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Introduction

Over the past decades, the incidence of cancer has increased constantly.¹ The World Health Organization estimates that by 2030, 1.4 million women of reproductive age will be newly diagnosed with cancer each year.² In addition, therapeutic progress has improved the survival rates of children and young adults with cancer significantly through treatments which are potentially deleterious for reproductive function.¹ The mechanisms by which chemotherapies induce ovarian damage are only partially known³ as each type of drug appears to affect the ovaries through different processes. In addition, other factors, such as the patient’s age and ovarian reserve, also contribute to residual ovarian function following the administration of gonadotoxic agents. Therefore, a new population of young adults, survivors of cancer and exposed to the detrimental side effects of treatments, has emerged in recent years and will continue to increase. The remarkable advances in cancer treatment have led to rethinking the management of malignancies in young patients. Indeed, the quality of life after healing has become a major issue. In women of childbearing age, fertility is in the foreground.⁴ Therefore, specialized consultations regarding oncofertility are recommended before gonadotoxic treatment for all women of reproductive age.⁴ Several female fertility preservation (FP) techniques are available and could be

proposed to young patients facing cancer.⁵ Nevertheless, few live births have been reported in women having used FP in oncological contexts. Thus, improving the FP methods currently available, developing new FP strategies, and better informing patients about fertility after cancer represent major challenges in oncofertility.

Anti-Müllerian hormone (AMH; also known as “MIS” for “Müllerian Inhibiting Substance”) is a member of the transforming growth factor (TGF)-beta superfamily whose primary physiological role in mammals occurs during embryogenesis. It was discovered in 1947 by Alfred Jost, who was the first to suspect the existence of a specific factor influencing male sexual differentiation during embryonic life.⁶ The *SRY* gene, expressed on chromosome Y, is responsible for testicular differentiation, that is, the Müllerian ducts regress under the influence of AMH (produced by Sertoli cells), while Wolffian ducts are maintained under the influence of testosterone (produced by Leydig cells). In 1984, Vigier et al⁷ discovered that AMH was also secreted in women. Produced exclusively by the granulosa cells of small, growing follicles, it is detectable in humans from 36 weeks of gestation until menopause. Although released into the blood and detectable in plasma, its physiological activity is essentially ovarian. It is involved in the regulation of the recruitment of primordial follicles and in follicular growth.⁸



Anti-Müllerian hormone assays were developed for women in 1990s,⁹ and the serum AMH level has rapidly become a factor in a wide array of clinical applications, based mainly on its ability to be a quantitative marker of ovarian reserve.⁹ The AMH level is very useful in reproductive medicine, particularly for the prediction of the ovarian response to controlled ovarian stimulation (COS) before in vitro fertilization (IVF), offering the possibility of individualized counseling and adjustments of the stimulation regimen.

The present article aims to review the potential utility of AMH in the field of oncofertility. Indeed, the serum AMH level can be used as a blood marker to inform patients of their expected ovarian function following gonadotoxic treatment, but it also plays a key role in guiding FP strategies. Moreover, this hormone could potentially be used as a new therapeutic option to limit chemotherapy-induced ovarian damage.

Clinical Implications of Serum AMH Levels for FP

Serum AMH Level: A Marker of Ovarian Reserve

AMH is secreted by small follicles as soon as they leave the quiescence phase until they reach the small antral stage.¹⁰ This secretion profile suggests that the AMH level could reflect ovarian reserve, as the initial follicular recruitment appears to be continuous over the course of a woman's reproductive life and proportional to the number of primordial follicles remaining in the ovaries.¹¹ It has indeed been shown that serum AMH measurement is correlated with the number of small primordial follicles both in women^{12,13} and mice.¹⁴ In addition, it has been established clearly that serum AMH levels are strongly correlated with an ultrasound evaluation of the number of small antral follicles, a procedure which is recognized as another excellent marker of ovarian reserve.^{15,16}

Serum AMH measurement offers many advantages over more conventional hormonal markers of the ovarian reserve, such as follicle-stimulating hormone (FSH), estradiol, or inhibin B. In particular, it appears to be relatively constant over the menstrual cycle, despite some studies reporting minimal and probably insignificant variations in clinical practice.¹⁷ Thus, it has been recognized for several years as the best hormonal indicator of ovarian reserve¹⁸ even though it fails to predict fertility or oocyte quality.¹⁹ Nevertheless, as the secretion of AMH is controlled by many factors, the results of this assay must be interpreted according to the clinical context.⁹

The clinical applications of serum AMH levels in women are numerous. First, it is strongly correlated with oocyte yield in assisted reproduction. Many studies have shown that the serum AMH level is the best predictor of ovarian response to IVF stimulation, which can be variable among women.^{20,21} Nevertheless, its ability to predict pregnancy and live birth after assisted reproduction has not been demonstrated.^{22,23} Moreover, AMH has become an additional tool for diagnosing premature ovarian insufficiency (POI) or polycystic ovarian syndrome (PCOS).²⁴

The ability of AMH to reflect ovarian reserve in women facing cancer has been challenged, as several studies have reported that some women with cancer could have reduced AMH levels compared with healthy women, even before the beginning of gonadotoxic treatment.^{25,26} However, other studies have failed to find any significant difference between the AMH levels of women with cancer and controls.^{27,28} Thus, the actual impact of cancer on ovarian reserve and function remains to be determined. Nevertheless, serum AMH determination at the time of cancer diagnosis could still have a major role in informing patients and helping both patients and clinicians to make decisions regarding FP strategies. Moreover, AMH assessment during treatment and after healing may also be helpful in predicting ovarian function, but the results should be interpreted with caution.

Serum AMH Levels for FP Counseling

Using AMH to predict ovarian reserve after cancer management. According to several studies, serum AMH levels before cancer could represent a relatively good prognostic marker of ovarian reserve after completion of treatment.²⁹ Indeed, it has been shown that women with higher pretreatment AMH levels had higher postchemotherapy levels,³⁰ whereas lower prechemotherapy values were associated with a slower rate of recovery in AMH levels.³¹ Moreover, further investigations in breast cancer patients have demonstrated that AMH measurement before treatment, in association with age, could increase the accuracy of prediction of cancer-therapy-related amenorrhea significantly.³² These results could help to better inform patients about ovarian function after cancer therapy and allow patients to decide whether or not to undergo FP techniques based on their individualized risks of POI. Nevertheless, although some studies have revealed that AMH levels could predict ovarian function and ovarian reserve after chemotherapy, an individual susceptibility to gonadotoxicity might exist. Indeed, in a recent study, Decanter et al³³ showed that in very young breast cancer patients with the same ovarian reserve parameters and receiving the same treatment protocol, different patterns of ovarian recovery (evaluated by ultrasensitive AMH assays) after chemotherapy are possible. Thus, predicting ovarian reserve after healing remains a huge challenge. Moreover, it is essential to inform patients that there is no evidence that precancer or even postcancer treatment AMH levels can predict posttreatment fertility, as pregnancies can occur even with an undetectable AMH serum level.³⁴

AMH to guide the choice of FP technique. Beyond representing an interesting tool with which to predict ovarian reserve after chemotherapy, the AMH level can also guide patients and physicians in choosing the best FP technique via its linkages with the available outcomes of different FP techniques.

Oocyte or embryo vitrification after COS is currently the gold standard FP technique and should be proposed when possible.⁵ In conventional COS for IVF, the AMH level represents a good marker of ovarian response in cancer patients.

Nevertheless, controversies exist about the impact of malignancies on ovarian response to COS. In healthy patients, recent data have suggested that 8 to 20 mature oocytes after COS are needed to achieve a reasonable success of live birth, but this number should be individualized according to age.³⁵ Data on pregnancies obtained after use of vitrified oocytes before cancer are still scarce.³⁶ The chances of pregnancy are strongly correlated with age at the time of FP as well as the number of mature oocytes obtained.³⁷ Thus, patients should be informed that even if the AMH level cannot predict a live birth after use of cryopreserved oocytes/embryos, it is related closely to the number of potentially vitrified oocytes, and it can help to determine the initial stimulation dose of recombinant FSH.

In vitro maturation (IVM) of cumulus oocyte complexes (COCs) followed by oocyte cryopreservation has recently emerged as an option for urgent FP or when ovarian stimulation is contraindicated. This technique is still considered to be experimental. It has been reported that the number of mature oocytes after IVM is highly associated with serum AMH levels.^{13,38,39} In healthy patients, data indicate that 8 to 20 cryopreserved oocytes after COS maximize the chance of obtaining a live birth.^{35,40} Nevertheless, data obtained from infertile PCOS women have shown a reduced competence of IVM oocytes when compared with oocytes recovered after COS.⁴¹ Moreover, the potential of cryopreserved IVM oocytes from cancer patients remains unknown. As a consequence, the optimal number of IVM oocytes frozen in candidates for FP is currently unpredictable. However, it seems that this technique should be considered for FP only when ovarian stimulation is unfeasible, particularly when markers of the follicular ovarian status are in a relatively high range.³⁸ Indeed, we showed that high values of AMH and antral follicle count (AFC) were needed for freezing a significant number of in vitro mature oocytes.³⁸ In this study, a threshold of 20 antral follicles and 3.7 ng/mL AMH were deemed necessary for the cryopreservation of at least 10 MII oocytes after IVM. In contrast, for a cancer patient presenting with a low to normal ovarian reserve, the best strategy to be proposed in the case of urgent FP must be considered.

Ovarian cortex cryopreservation and transplantation (OCT) is another experimental FP technique available when oocyte or embryo vitrification after COS cannot be performed. A recent study suggests that the serum AMH level is the best predictor of primordial follicle density in a population of young women with presumably healthy ovaries and may thus represent a reliable marker of the pool of nongrowing follicles.¹³ This correlation between AMH and primordial follicle count was also reported in other studies.^{12,42,43} The current recommendations propose that women older than 35 years of age should not be considered candidates for OCT due to both low ovarian reserve and reduced oocyte quality.⁴⁴ However, to date, no data have been reported on the effectiveness of ovarian tissue graft in young women who have reduced ovarian reserve, so no reliable conclusions can be drawn.

Potential Therapeutic Clinical Applications of AMH

Chemotherapy induces a decrease in fertility by exerting direct toxicity on the ovaries. It leads to a depletion of the oocyte stock through several possible mechanisms that are still being debated. Recently, a new mechanism for follicular depletion induced by cyclophosphamide, called the “burn-out effect,” has been proposed and consists of a massive growth of resting follicles, which are then destroyed. As AMH is one of the factors regulating the recruitment of primordial follicles from the ovarian reserve, it could be used in a new treatment to limit follicular depletion induced by chemotherapy.

Background

Mechanisms of chemotherapy gonadal toxicity. During chemotherapy administration, drugs induce, almost systematically, the destruction of growing follicles, resulting in transient amenorrhea occurring rapidly after the first cycles of chemotherapy. This effect may be transitory, as evidenced by the possibility of normal ovarian function after the completion of treatment.⁴⁵ However, chemotherapy protocols may induce a more or less significant alteration in the follicular stock. The depth of follicular depletion is largely dependent on several factors, such as the type and dose of molecule used as well as the patient’s age and ovarian reserve at the time of treatment.⁴⁶

Mechanisms to explain the gonadotoxicity of these different molecules have been explored in various experimental models, ranging from histological studies of female ovaries after chemotherapy to cell cultures in the presence of active metabolites of chemical agents. Moreover, animal models have been used widely to understand the effects of different cytotoxic agents on ovarian function. A large number of studies have been carried out, and several hypotheses have been raised.³

The most commonly accepted theory is based on a direct alteration of the DNA of oocytes contained in primordial follicles resulting in follicular atresia.⁴⁷ Double strand breaks represent the main DNA lesions caused by cytotoxic agents. These DNA alterations can lead to either DNA repair pathways allowing cell survival or cell death by apoptosis. According to this theory, chemotherapy induces follicular depletion by affecting the primordial follicles that undergo atresia directly.

More recently, it has been suggested that some chemotherapies, such as cyclophosphamide or cisplatin, may induce simultaneously a massive growth of primordial follicles and apoptosis of growing follicles.^{48–50} According to this theory, the drugs induce recruitment of primordial follicles through the activation of the PI3K pathway, known to be essential for primordial follicle resting.^{51,52} This concept of ovarian reserve depletion is called the “burn-out effect.”⁴⁸ This model could also explain the alteration of the ovarian reserve induced by the presence of an ovarian endometrioma⁵³ or massive follicular loss secondary to ovarian cortex transplantation.^{54,55}

Finally, histological analyses of ovaries after chemotherapy show endothelial vascular complications that may cause tissue fibrosis and, consequently, impaired ovarian function.⁵⁶

Better understanding of the gonadotoxicity mechanisms is essential, and the progress made thus far has led to the development of methods to limit the impact of chemotherapy on the ovaries.

Physiological role of AMH in ovaries. AMH is a glycoprotein hormone expressed by granulosa cells surrounding the oocytes. It is produced by follicles from the primary stage of development until selection for dominance¹⁰ and plays a key role during folliculogenesis. It was demonstrated in several experimental models that AMH is implicated in the inhibition of the recruitment of primordial follicles and in the regulation of the sensitivity of granulosa cells to FSH.

First, AMH seems to be involved in the initial recruitment of primordial follicles.⁵⁷ Indeed, the ovaries of *Amb^{-/-}* mice show both a decreased number of primordial follicles and an increased number of growing follicles compared with wild-type ovaries.⁵⁸ These results suggest that AMH could act as a major factor in the regulation of primordial recruitment. Moreover, *in vitro* experiments on human,⁵⁹ bovine,⁶⁰ and rodent ovaries^{61,62} have revealed that the transition from primordial into growing follicle was improved in the absence of AMH, whereas this activation was blocked when AMH was added to the culture medium. Another experimental model, using a graft of a mouse ovarian cortex onto a chicken embryo containing high levels of AMH, confirmed this hypothesis.⁶³ Indeed, the recruitment of primordial follicles was decreased when the mouse ovarian cortex was inserted into the chicken embryo. However, primordial follicles were recruited at a high rate after the transplantation of *Amhr2^{-/-}* mouse ovarian cortex or when the graft was performed on a gonadectomized chicken without endogenous AMH.⁶³

AMH seems to also be involved in the regulation of follicular sensitivity to FSH and in the selection of the dominant follicle.⁶⁴ It was shown that the proliferation of antral follicle granulosa cells, induced by FSH, was inhibited by the addition of AMH.⁶⁴ Moreover, AMH reduces the synthesis of P450 aromatase, whereas this synthesis is induced by FSH.^{65,66} In addition, in human luteinized granulosa cells, AMH decreases the expression of the *CYP19a1* gene encoding aromatase and, consequently, the production of estradiol.⁶⁷

Other roles of AMH such as antiproliferative action on different malignant ovarian cell lines have been reported.⁶⁸ This antiproliferative action has also been demonstrated in other organs expressing the AMH receptor, such as breast, uterine, and endometrial cells.⁶⁹

Another unexplored effect of AMH on ovarian function is its potential involvement in pituitary function, thus potentially assigning it a secondary effect on growing follicles. This impact of AMH on the hypothalamus and pituitary is newly demonstrated and needs to be confirmed.⁷⁰

AMH to Preserve Ovarian Reserve

Currently, in the field of oncofertility, efforts are constantly being made to improve existing FP techniques and to develop treatments to limit follicular depletion *in vivo*.^{3,45} To date, these therapies have nearly all reached the fundamental research stage.

Because AMH has been shown to limit the activation of primordial follicles in *in vivo* or *in vitro* mouse models,^{58,62,71} it was suggested in 2 recent studies that this hormone could be an effective treatment in terms of limiting chemotherapy-induced gonadotoxicity. This hypothesis was supported by the expression of the specific AMH receptor, *AmhrII*, in mice follicles following in the primordial stage.^{62,72}

Kano et al⁷² reported that, in mice, superphysiological doses of AMH, delivered either by recombinant protein via osmotic pumps or a gene therapy, could decrease primordial follicle loss induced by cyclophosphamide, cisplatin, or doxorubicin, chemotherapies used currently in oncology. The protective effect of AMH varied between drugs and was stronger for carboplatin than for cisplatin or cyclophosphamide. These data suggest different mechanisms for ovarian damage which may depend on the toxicity of each drug to granulosa and/or germ cells. In addition, this study revealed that AMH could also represent a new contraceptive method, as continuous daily exogenous administration of AMH in mice induced an alteration in estrial cycles and a drastic decrease in fertility in a dose-dependent manner. These data are in accordance with the role of AMH in FSH sensitivity.⁶⁴

Recently, we performed a study to assess this protective effect of AMH in pubertal mice treated with cyclophosphamide.⁷³ In our model, we first confirmed the inhibitory role AMH plays in the recruitment of primordial follicles, as, 1 week after a single administration of AMH, the proportion of primordial follicles increased, contrasting with the decrease in the proportion of early-growing follicles. Second, we showed that in the ovaries of mice treated with concomitant injections of cyclophosphamide and AMH, the number of primordial and early-growing follicles were similar to what was observed in the controls, whereas the ovaries of cyclophosphamide-treated mice were depleted of primordial follicles, suggesting that AMH effectively protects the follicular stockpile from cyclophosphamide-induced damage. Next, we evaluated the long-term effect of these treatments on ovarian function and fertility. We showed, via mating experiments, that 15 weeks after the end of the treatment, the cumulative number of pups was slightly greater in mice having received both treatments as compared with cyclophosphamide alone and that the number of ovulated eggs after ovarian stimulation was significantly reduced in Cy-treated mice and rescued by AMH coadministration. Overall, in this pubertal mouse model, our results provide new evidence that AMH inhibits the recruitment of primordial follicles and could therefore protect mice from cyclophosphamide-induced gonadotoxicity.

If these results are confirmed and approved in women, recombinant AMH could be a new option for preserving the fertility of women who require alkylating treatment. Currently, several “fertoprotectant” molecules, such as rapamycin or melatonin, have been developed and studied in terms of their abilities to limit chemotherapy-induced ovarian damages in mice.^{45,74,75} Nevertheless, these treatments are involved in ubiquitous signaling pathways or apoptosis; therefore, they could interfere with physiologic mechanisms or the efficacy of chemotherapy. As AMH is produced only by ovaries and acts through a specific receptor expressed mainly by the ovaries, this hormone might be a particularly interesting new agent in terms of protecting the ovarian reserve and subsequent fertility as it acts as a targeted therapy.

Recombinant AMH has also been used to improve the results of ovarian tissue cryopreservation, a still experimental, but promising, FP technique.⁵ The autograft of cryopreserved ovarian tissue have resulted in a live birth in a patient healing from cancer and allowed the restoration of hormonal function in a young girl.⁷⁶ Nevertheless, it has been shown that the manipulation of the ovarian cortex after removal induced a large loss in primordial follicles, possibly via a burn-out effect.^{54,55} Consequently, *in vivo* or *in vitro* AMH treatment could protect the follicular stockpile under these conditions and improve the FP technique.⁷⁷ Indeed, this treatment, which allows the inhibition of initial primordial follicle growth, could preserve quiescent follicles in the ovarian cortex and ameliorate the ovarian graft function and duration.

Thus, all these data have revealed that AMH might represent a new treatment for limiting primordial follicle depletion induced by chemotherapy, but these encouraging results need to be confirmed.

Other Roles of AMH in Oncology

The primary role of AMH is to induce the regression of the Müllerian ducts during embryonic development. As most genital tract tumors derive from these structures, it has been proposed that AMH may inhibit the growth of some gynaecological tumors.⁶⁹ By acting only on cells expressing the AMH receptor, this molecule could act as a targeted therapy.^{78,79}

AMH was first proposed as a potential therapy in ovarian cancer. It has been shown in rodents that AMH could induce the proliferation inhibition and apoptosis of ovarian epithelial cancer cells *in vitro* and *in vivo*.⁸⁰ These results appear to be transposable in women, as human cancer ovarian cells express AMHR2 and their *in vitro* growth is inhibited by recombinant AMH.⁶⁸ Endometrium and the uterine cervix also express AMHR2, and *in vitro* studies have demonstrated this antiproliferative role of AMH in human endometrial or cervical cancer lines.^{81,82} Finally, weak expression of AMHR2 has also been documented in human breast tissue both in physiological and pathological conditions, and AMH also appears to have an antiproliferative effect on breast cancer cell lines.⁸³

Conclusion

In conclusion, recombinant AMH may represent a new targeted and innovative therapy with a large spectrum of applications in oncology and FP. However, to date, this molecule has never been tested in women, and extensive additional studies, both fundamental and clinical, are necessary. If these results are confirmed in women, it seems possible that recombinant AMH could represent a future treatment for FP during chemotherapy. Nevertheless, the indication, the mode, and the duration of administration as well as the potential secondary effects of this molecule must be evaluated. Thus, the recent developments summarized in the review have provided specific research questions on the role of AMH. Moreover, future clinical trials should be performed to better evaluate and understand the use of AMH in oncofertility.

Author Contributions

CS wrote the manuscript, IB, NB and MG carefully revised the manuscript.

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